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APPLIED BIOCHEMISTRY

By

WITHROW MORSE, Ph.D. (Columbia)
Professor of Physiological Chemistry and Toxicology,
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SECOND EDITION

Revised and reset with the co-operation of

JOSEPH M. LOONEY, M.D. (Harvard)
Assistant Professor of Physiological Chemistry, Jefferson
Medical College, Philadelphia

Dii laboribus omnia vendunt

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To

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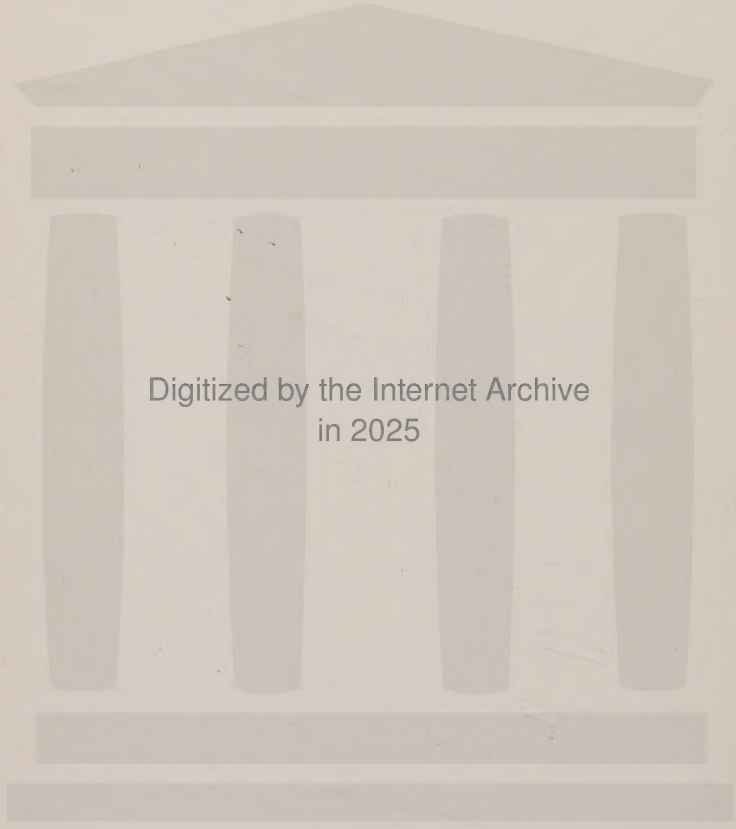
Physicist, Teacher, Friend.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"I know of nothing that is so conducive to cheerful optimism as the pursuit of science. The laboratory is the habitation of buoyancy, enthusiasm, and hope. Its occupant has no moral right to be despondent, and if he is so, there is surely something pathological in the activities of his brain-cells. Actually, however, one rarely meets with a pessimistic man of science."—Lee, Columbia University.

"Science owes a great deal to the reasoning power of the thinker and to the acumen of the guesser, but both alike are futile until facts are actually determined." —Halliburton, Kings College, London.

"The laboratory is the fore-court of the temple of philosophy; and whoso has not offered sacrifices and undergone purification there, has little chance of admission into the sanctuary."—Huxley, English physiologist, 1825–1895.

"The ultimate object of all medical research is practice. In the final analysis the test of the value of any research is to be found in the application of the results. —Patterson, Dean, Jefferson Medical College.

"The hospital represents what we know; the laboratory what we hope to know." —Trudeau, Founder of the Adirondack Sanitarium.

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PREFACE TO THE SECOND EDITION

THROUGH the courtesy of the publishers, an extensive revision of this book has been made possible. It is a favorable commentary on the science that within eighteen months of the publication of the first issue, much new and imperatively important material must be included in a new edition if this issue is to be brought to date. Through the coöperation of friends and colleagues, especially Professor Looney, Dr. Brubaker, Dr. Van Slyke, Dr. Geiling, Dr. Jules Bebie, Professor Sumner, Dr. B. B. V. Lyon and others, space for whose names is unavailable, it is possible to correct unfortunate errors and to include important developments: the more modern concepts in physical chemical physiology, such as the theory of the nucleus of the atom, the concept of activity, oxidation—reduction phenomena and methods, especially of the quinhydron electrode, the molecular weights of protids, revision of the amino-acid content of protids, more exact analytical procedures such as methods for amino-acids; the exact, rapid and convenient methods devised by Van Slyke for manometric determinations, etc. A complete and modern scheme for analysis of urinary calculi is included. The rapid development of the studies in the metabolism of calcium has demanded the enlargement of this subject and especial treatment of subject matter and methods. Newer discoveries on the relation of guanidin and sugar metabolism in the so-called "artificial insulin;" the presentation of rational formulae for mucoprotids (P. A. Levene), for the starches (J. C. Irvine), etc., are presented for the first time in a text-book for medical students. It is hoped that the present edition will meet the favorable reception given the first issue.

W. M.

1001 WALNUT STREET,
PHILADELPHIA, PA.,
September, 1927

PREFACE

The following pages have been written with a view to weaving the woof of biochemistry into the warp of medicine. The student of general chemical physiology already has at his command excellent treatises, like that of Mathews, the first modern text-book of biochemistry. There remains the task of providing the medical student with the biochemical facts and principles which bear more or less directly upon his science and art. Each of the sciences contributory to medicine tends to grow until, like the fabled camel, it nearly fills the curricular tent. It is essential, therefore, if the curriculum is to remain well-balanced, that each department present only such material as is definitely related to medicine as a whole. This book aims so to present biochemistry.

Theory and practice are intermingled in all science. Facts are the bricks and theory is the mortar with which the edifice of biochemistry has been erected.

No apology is offered for the somewhat detailed and exhaustive treatment of many subjects. Even the casual student becomes restless with mere mathematical factors and incomplete explanations. The theory of Benedict's method for the detection of sugar may not be essential to diagnosis, but it leads to a clearer understanding and satisfies the inquiring mind.

"The gods sell everything for labor."[1] Unless the present generation becomes parasitic upon the past, it must contribute to the development of science. To that end the student of biochemistry, whether he intends to be a theoretical or a practical worker, must, like Ulysses,

> "Follow knowledge like a sinking star
> Beyond the outmost bound of human thought."

That is all there is to the problem of research. It must ever be a by-product in the work-a-day world of the physician, but, like the tar

[1] This inscription stands over the portal of Jefferson Medical College; see title page.

of the coal, the wastings may become the most valuable part of the yield.

Science is a mosaic, the parts of which have been made by the labors of thousands of workers. Text-books are largely compilations and it is impossible to assign credit where credit is due. Nevertheless the author is unwilling to close the book without expressing his gratitude to colleagues and friends:

To Professor Albert P. Brubaker he is indebted for many exceptional courtesies, not the least of which is the privilege of using the magnificent library of the College of Physicians as well as Dr. Brubaker's private books and separata.

The officers of the American Society of Biological Chemists Inc. have permitted the publication of their portraits. This favor will enable the student to visualize the men who are contributing to the advance of biochemistry. While science knows no international boundaries, American workers are naturally of signal interest to the American student.[1]

The Arthur H. Thomas Company has been of great assistance in many ways. The reproductions of standard biochemical apparatus published herewith are largely those provided for the purpose by this house.

Finally, the author expresses his appreciation of the patient consideration given him by the W. B. Saunders Company.

The manuscript was read by Mr. J. S. De Frates[2] of the Department of Physiological Chemistry, Jefferson Medical College, and edited by Miss Esther G. Price of New York City. For these painstaking labors the author is most grateful.

THE AUTHOR.

[1] The photograph of Carl Voit, Fig. 179, page 595 was loaned by Professor Brubaker.

[2] Harvard University, Fellow in Biochemistry, 1925–1926.

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APPLIED BIOCHEMISTRY

INTRODUCTORY

Biochemistry is a science.[1] Huxley defined science as "Knowledge set in order." It is not merely facts. We know that a sulphurous odor arises when hair is burned; that hair grows from the body, and that the body receives all its substance from food. If we organize this knowledge, we may assume that sulphur occurs in our food. This is, however, merely an hypothesis and must be verified by chemical analysis before it becomes a part of science. The more perfect science becomes, the greater the surety that our predictions (hypotheses) are correct.

Acquiring Knowledge.—The human body may be compared to a ship upon the sea. Both the body and the ship have mechanisms for receiving impressions from the external world. Man has his photo-receptors (eyes), phono-receptors (ears), and other specialized organs. The ship carries binoculars, fog-horn and submarine bell receptors, aërials for wireless messages, and sextants for determining location. Impressions are gained by these instruments. On the ship they are entered into the log and are interpreted by the commanding officer. In the body they are transmitted to the brain and interpreted by it; the brain is the commanding officer. Until facts are interpreted, that is, correlated with other facts, they do not form a part of science.

Different Kinds of Knowledge.—When we organize facts we find that there are different classes of knowledge. One kind concerns living things, while another deals with lifeless objects and processes. Knowledge of living things forms the science of biology,[2] of their form, morphology,[3] and of their actions, physiology.[4]

Physical and Chemical Physiology.—The science dealing with the activities of living things may be considered from the standpoint of the mechanics of movements, of the flow of body fluids, and of the

[1] Latin *scio*, I know.
[2] Greek *bios*, life, and *logos*, a discourse.
[3] Greek *morphe*, form.
[4] Greek *physis*, nature or character.

passage of impulses through the nerves. Such considerations demand a knowledge of physics and may be designated as "physical physiology" or "bio-physics." On the other hand, we may be interested in the substances of which the body is composed and the manner in which these substances undergo transformation in the organism. This brings us into the field of chemistry and we term such studies "chemical physiology."

In chemical physiology, or, as it is usually termed, "physiological chemistry," we have a blending of biology and chemistry and for this reason the science is frequently designated "biochemistry." The terms "chemical physiology," "physiological chemistry," "biological chemistry," or "biochemistry" are all synonymous.

Biochemistry and Chemistry.—For the medical student who aims to utilize chemistry in his studies of the human being in health and in disease, chemistry is a means to an end and not an end in itself. Chemistry must be the tool for the study of the structure and functions of the body as physics is the instrument whereby the physiologist determines the flow of the blood and the movements of muscles and bones. Thus, biochemistry will advance as new facts and principles are developed in pure chemistry.

Medicine and Biochemistry.—Medicine aims to prevent or cure faulty action of the body and biochemistry is concerned with those actions which are interpretable in chemical terms. It is evident, then, that the two sciences are closely interrelated. The physician must understand the chemical processes of digestion; thereby he may learn the normal way in which foods are prepared for use in the body. He must know the chemical composition of body fluids; it is through them that foods are distributed and the waste is gathered from the organs. He must know the chemical composition of the urine; in this fluid the excretions of the body are largely disposed. By learning the normal processes of the body one is enabled to deal with the abnormal or pathological conditions. While practically all sciences have contributed to the great advances of medicine, chemistry holds an important place. Urinalysis is necessary in clinical medicine. Serious diseases, like diabetes mellitus, nephritis, and goiter, are influenced and sometimes controlled by chemical means. Surgery is dependent upon chemistry for aid in diagnosis and treatment. Every where the physician and the surgeon appeal to the chemist for assistance.

The Instruments of Biochemistry.—Science is divided into branches principally because special means must be employed for

study in certain fields. For example, astronomy deals with large but remote objects, whereas chemistry is concerned with minute but tangible substances. Hence the study of one requires the telescope, and of the other, the instruments of the chemical laboratory. Likewise, biochemistry and physiology each require special types of instruments. The physiologist utilizes apparatus to record elapsed time for the study of muscle contraction and for timing the passage of a nerve impulse. The biochemist uses the balance, the spec-

Fig. 1.—Otto Folin, Hamilton Kuhn Professor of Biochemistry, Harvard University Medical School, Boston; originator and exponent of colorimetric methods for rapid determinations of important biochemical substances, investigator and inspirer in many lines of fundamental biochemical research.

troscope, and other instruments. The peculiar nature of living substance requires procedures and apparatus which differ from those used by the general chemist.

Biochemistry in America is especially characterized by the invention of methods. Folin,[1] Benedict,[2] Van Slyke,[3] Myers,[4] and others have devised methods which greatly shorten the period necessary

[1] Otto Folin, Professor of Biochemistry, Harvard Medical School, Boston, Massachusetts (Fig. 1).

[2] S. R. Benedict, Professor of Biochemistry, Cornell University Medical College, New York (Fig. 5, page 31).

[3] D. D. Van Slyke, Member of Rockefeller Institute, New York (Fig. 34).

[4] V. C. Myers, Professor of Biochemistry, Western Reserve University Cleveland, Ohio (Fig. 236).

for making determinations and which also require small amounts of material. It is a routine procedure for the clinical chemist to report within two hours after the blood is drawn from the patient the following quantitative determinations made upon 10 mls. of blood:

	Mgs. 100 mls. blood.
Non-protid nitrogen, normal amount	30.9
Urea nitrogen	14
Uric acid	3.4
Creatinin	1.5
Chlorides as NaCl	500
Sugar	100

The degree of accuracy varies with the chemist, but the error may be under 0.01 mg. per 100 mls. blood.

The Aim of Biochemistry.—This may be stated in the words of an eminent British chemist: "In the not far distant future the whole subject of biochemistry, which is the chemistry of life, will form but a part of organic chemistry, and we shall be able to apply to it the means and methods which have been attended with such astounding success during the past sixty years in the treatment of the parent science. When this is the case, the causes of our bodily ills will be as clear as the structure of indigo; and their removal as easy as a test-tube reaction. The treatment of disease will be as sure and as certain as the neutralization of ammonia by sulphuric acid."[1] If this statement seems extravagant, let it be recalled how considerably the length of life in diabetes mellitus has been prolonged since the introduction of insulin after its discovery by the Toronto group,[2] and that this decrease in mortality has occurred in spite of a material increase in the incidence of the disease. Again, owing to the discovery of various chemical substances, such as the arsenical compounds (salvarsan, etc.), the dread disease syphilis, responsible for the terrible conditions during the whole of human history, and more particularly since the discovery of America by Columbus, has been brought under control by medical treatment.[3] Lastly, we should remember how

[1] J. S. Thorpe, Imperial College, London. See Armstrong in Suggested Readings, page 21.

[2] Banting, the discoverer of the means by which the pancreatic extract can be obtained without destruction; Best, his collaborator; Collip, who devised the method for quantity production, and Professor J. J. R. MacLeod, the head of the department in which the work was done. See Joslin's treatise on Diabetes for statistics concerning the use of insulin.

[3] Read Huxley's contrast of his time and that of the founders of the Royal Society of London in "Methods and Results," article Progress of Science.

much we owe the completion of the Panama Canal to the chemical means of sanitation and the quinin treatment of malaria.[1]

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THE ORIGIN OF AMERICAN BIOCHEMISTRY

Biochemistry a Young Science.—Biochemistry is a youth among the sciences. Its beginnings lie far in the past, if we ascribe to the making of wine from grapes,[2] the making of vinegar,[3] and similar phenomena of enzyme nature evidence of the dawn of biochemical knowledge. To the French, however, we must turn for the foundations of biochemistry as a science proper; for the closing years of the 18th Century witnessed alike the epoch-making generalizations of Lavoisier[4] and his untimely death in the Reign of Terror. Associated with Lavoisier was Pierre Simon Laplace, who carried the investigations into the Nineteenth Century. Together they founded the essentials of the energetics of the body[5] and laid the foundations for the modern principles of nutrition and the methods of diagnosis of certain diseases.

The Foundations as Biochemistry.—It is easy to remember the date of the birth of biochemistry as a science, for it falls at the beginning of the Nineteenth Century. The mantle of these French savants

[1] The impotence of science, after discovering the means of relieving human ills in the face of political events, is well illustrated by the plague of malaria devastating eastern countries, while at the same time a great state producing quinin is advertising elaborately for markets for her product.

[2] Genesis, Chapter 9, verse 21.

[3] Numbers, Chapter 6, verse 3.

[4] Antoine Laurent Lavoisier. For picture see Bayliss's General Physiology.

[5] For a discussion of these principles see Chapter XIII.

fell somewhat later upon Justus von Liebig, from whom American biochemistry may be assumed to have been derived; for from the first chemical laboratory[1] ever founded, that at Giessen, in Germany, by Liebig after his student days in Paris under disciples of Lavoisier, and later from Liebig's Munich workshop have descended several students of biochemistry who laid the foundations of that science in America. Among these may be mentioned Chittenden,[2] who indirectly received the influence of the Munich school and who founded the Yale school of biochemists. Many of the important posts in various institutions of this country are held by students of Chittenden or his followers. A quarter century ago William J. Gies and colleagues opened the first teaching department of biochemistry in America at the College of Physicians and Surgeons, now a part of Columbia University, New York. It would be impossible to follow the development of the various groups of biochemists in this chapter, and recourse is made to a chart[3] to illustrate some of the lines in the history of American biochemistry. In addition to the Yale group we have the students of Otto Folin and of Mandel of New York University. A somewhat different line is that derived from the Munich School of Nutrition under Carl Voit,[4] which is represented in this country by the late W. O. Atwater[5] and Graham Lusk.[6]

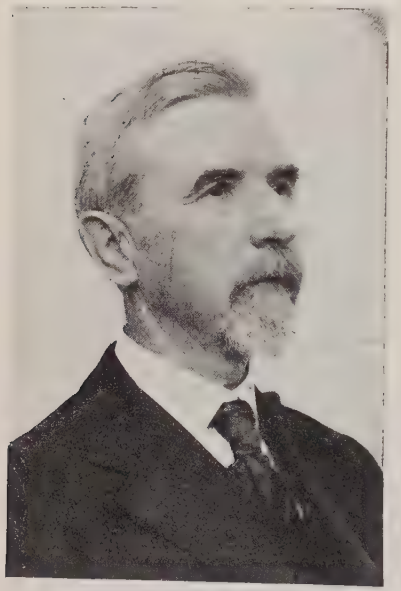


Fig. 2.—Russell H. Chittenden, Professor Emeritus, of Physiological Chemistry, Yale University. Researches bearing upon nutrition. Responsible for the training of many American biochemists.

[1] A laboratory was founded in Leningrad by Lomonossov before Liebig opened the Giessen laboratory, but the result was far less influential on chemistry than the German plant.

[2] Russell H. Chittenden, Emeritus Professor of Physiological Chemistry, Yale University, New Haven, Connecticut (Fig. 2).

[3] Page 24.

[4] Figure 179.

[5] W. O. Atwater, onetime Professor of Chemistry at Wesleyan University, Middletown, Conn. Later Atwater was associated with the Department of Agriculture at Washington. The first laboratory for the study of nutrition was built at Middletown. This was later moved to Washington, D. C., and its continuation is the Nutrition

The Development of Practical Biochemistry.—Throughout the world biochemistry has become an integral part of every hospital of importance. Sanatoriums, like that at Clifton Springs, New York;[1] special research foundations, like Rockefeller Institute[2] and Hospital,[3] and the Mayo Foundation of the University of Minnesota;[4] and the great metropolitan hospitals, like Roosevelt in New York,[5] the Presbyterian in Philadelphia[6] and others are centers of biochemical work. The importance of the subject in purely commercial institutions is made evident by the development of laboratories for routine and for research in such institutions as the pharmaceutical firm of Eli Lilly[7] and the life insurance company, the Metropolitan[8] of New York.

Periodicals on Biochemistry.—*Journal of Biological Chemistry*, published by the American Society of Biological Chemists, Inc. Address, the Williams and Wilkins Co., Baltimore, Md. This is the important medium for the dissemination of biochemical information in the United States ($5.00 per volume, about $20.00 a year).

Journal of the American Medical Association. Address Chicago, Illinois. Important editorials and abstracts covering especially applied biochemical knowledge are published in each issue (weekly, $5.00 a year).

Abstract Journal of the American Chemical Society, issued every fortnight. Contains reviews of most biochemical papers in all languages. Membership in the American Chemical Society ($15.00 per year) gives subscription without extra charge to three chemical journals: (1) Chemical Abstracts, (2) Journal of the American Chemical Society, and (3) Industrial and Engineering Chemistry. Biochemical articles appear in all of these magazines.

Laboratory of the Carnegie Institute of Washington; the laboratory is near the Harvard Medical Buildings, Boston, and its director is F. G. Benedict, a former associate of Atwater.

[6] Graham Lusk, Professor of Physiology, Cornell University Medical College, New York. Dr. Lusk is scientific director of the Russell Sage Institute of Pathology, New York, in which important studies in human nutrition have been conducted. The medical director is E. F. DuBois. See Chapter XIII.

[1] R. S. Hubbard, of the Folin Laboratory, biochemist.

[2] P. A. Levene is the Member in charge of chemistry (Fig. 95).

[3] D. D. Van Slyke is biochemist (Fig. 34).

[4] E. C. Kendall (Fig. 203), the discoverer of thyroxin (page 99), is chemist. The professor of biochemistry at Minneapolis is J. F. McClendon.

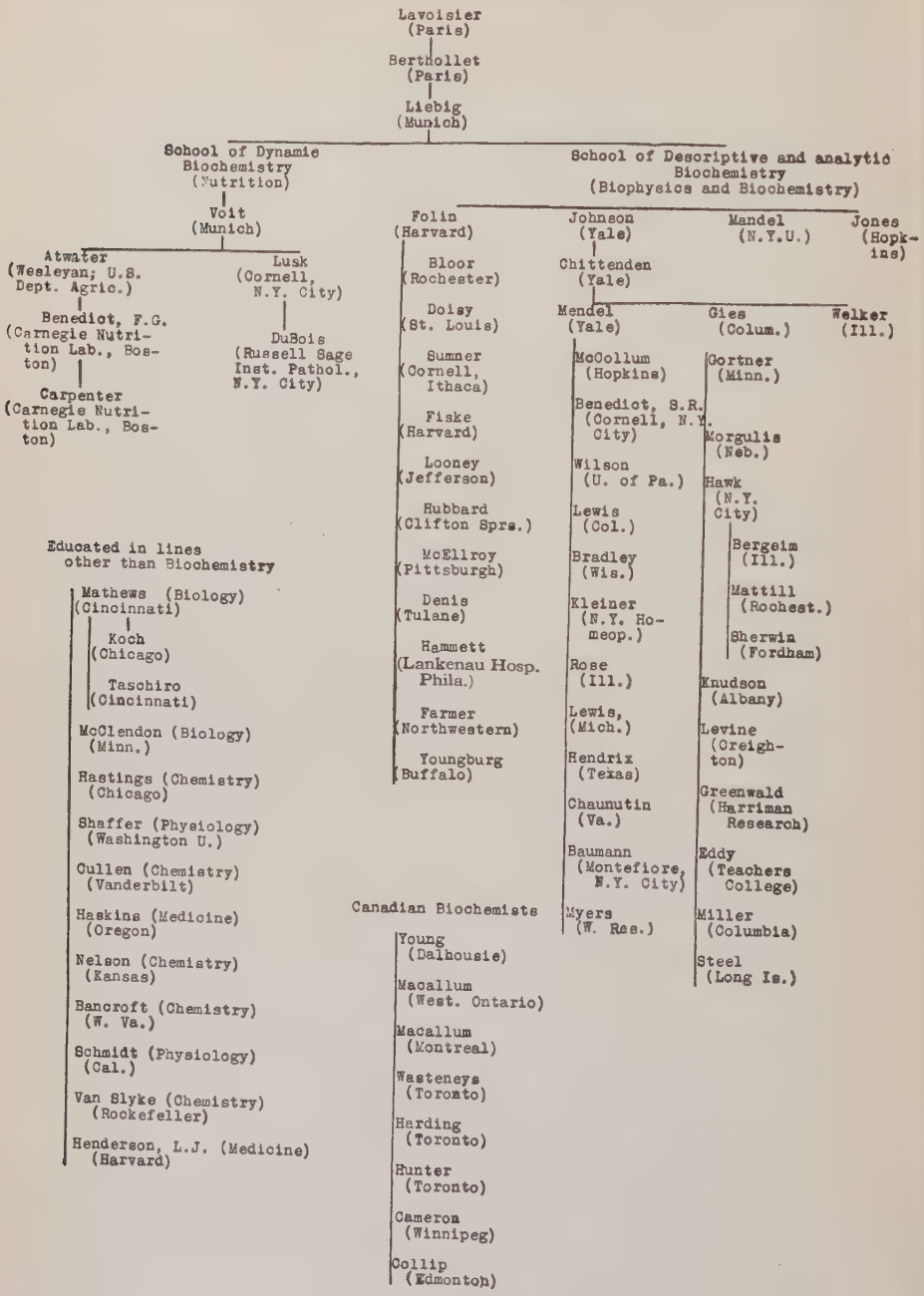
[5] A special department, the Harriman Research Laboratory, has as chemist K. G. Falk, and as biochemist, I. Greenwald.

[6] F. A. Cajori is biochemist.

[7] Eli Lilly & Co., Indianapolis, Indiana. The biochemist is G. H. A. Clowes.

[8] F. B. Kingsbury is biochemist.

ORIGIN OF AMERICAN BIOCHEMISTRY AND SOME OF THE LINES OF ITS DEVELOPMENT.



American Journal of Physiology. Address the editor, Johns Hopkins University, Baltimore, Md. While largely devoted to physical physiology, biochemical papers are published ($20.00 a year).

Proceedings of the Society for Experimental Biology and Medicine. Address the Secretary, A. J. Goldforb, College of the City of New York, New York. This is virtually a "Compte rendus" in which brief abstracts of important papers that appear later in some journal in full are published ($5.00 a year).

Biochemical Journal (England). Address Cambridge University Press, Cambridge, England, or the University of Chicago Press, Chicago, Illinois, U. S. A. This journal occupies in England and colonies the same place that the Journal of Biological Chemistry does in the United States. Issued every two months ($14.00 a year).

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CHAPTER I

MAN AND HIS ENVIRONMENT

"But a knowledge of the compositions of bodies is, after all, only the statical or secondary object of chemical inquiry; for, in common with physics, chemistry has primary reference to the varied existence of matter in time, and to the series of changes which it manifests."—*Odling.*[1]

THE human being as we see him today is the product of the action of forces and events which have molded him from beings quite unlike himself. The physician should bear this truth in mind. The chemical composition of the human blood[2] resembles that of sea-water or, rather, that of the sea-water of past ages. We know that animals which live in the sea incorporate sea-water into their bodies, and use it as blood is used in man. The correlation of these facts indicates that man's ancestors lived in the ocean,[3] and leads to the belief that the essential chemical composition of the water of the ocean is retained in the veins of man today. Mucin, a substance which is a typical component of the mucus or slime of the outer covering of the lower animals, is found in various parts of the human body. Within the nervous system of man and also in his hair and nails there is a chemical compounds, keratin, which commonly covers the body of lower forms. The apparent anomaly of finding such substances in the central nervous system is explained when we recall that this system originates from the outer layer, or ectoderm. Again, the teeth of man represent chemical compounds found in the protective coverings like the shagreen of the shark.

Various theories have been offered to explain the evolution of man from lower forms of life; of these, the theory of direct effect of the environment of Lamarck;[4] that of the survival of best-adapted varia-

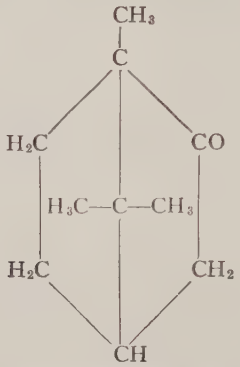
[1] Odling, W., English scientist of the 19th Century, lecturer, St. Bartholomew's Hospital, London.

[2] See Lotka, A., The Elements of Physical Biology, Baltimore, Williams & Wilkins, 1925, p. 201.

[3] Macallum, A. B. (McGill University, Montreal, Canada), Amer. Jour. Med. Sci., vol. 156, p. 1, 1918. Magnesium has increased in the sea-water since man's ancestors lived in that medium. (Percentage Mg in sea-water at present, 3.79 mgs.; in human blood, 2.3 mgs. (serum).)

[4] Jean B. Pierre Antoine de Monet Chevalier de Lamarck, eminent French natural historian of the 18th Century, supposed that the surroundings impressed themselves upon animals, and in time these effects became inherited.

tions of Darwin,[1] and the mutation theory of DeVries[2] may be mentioned. In the world of lifeless things we find illustrations of characteristics which are especially adapted to the substances bearing them. Thus, in camphor, two methyl groups lie in the middle of



the molecule in such a manner that they stabilize the structure, which would otherwise become unstable because of the methyl group at the top of the aromatic ring. Evidence of inorganic evolution is also found among the elements which will be discussed presently.

Man's Environment.—So far as we know man exists only upon the Earth. The Earth is one of many planets which revolve around the Sun, and comprise the Solar System. The Sun is but a star among thousands of stars belonging to the Universe, and our universe is but one of many. The astronomer considers the spiral nebulæ as other universes. It is well within the range of probability that beings similar to man exist in other worlds than ours. Indeed, it is possible that man exists on one of the planets, Mars.

Macrocosm and Microcosm.—Emerson[3] has spoken of the monotonous plan which nature uses in her work. The structure of the universe which we have just described is essentially duplicated in minature in the atom.[4] We may tabulate the corresponding points and then describe them:

[1] Charles Darwin, English naturalist of the 19th Century, suggested that all animals varied more or less from one another; the characteristics which are best adapted to the needs of the animal persist, others being lost.

[2] Hugo DeVries, formerly Professor of Botany, University of Amsterdam, The Netherlands, found that variations studied by Darwin arose suddenly in animals and plants, and that instead of small variations, frequently these changes were large and, if favorable, persisted. Read, however, modern criticisms by T. H. Morgan in various books published by him.

[3] Chapter VII, introductory.

[4] See Kramer, H. A., and Holst, H., The Atom and the Bohr Theory of Its Structure, New York, A. A. Knopf, 1924.

Solar system.

Sun, centrally located and containing much of the mass of the system; the total mass of the solar system is 1,002,681.89 and that of the sun alone, 1,001,341.00.

Planetoids and planets revolve about the sun, from which they are kept by their rapid movements, centrifugal force balancing the attraction of the sun. There is no exact similarity between their distribution and that of the electrons of the atom.

Atom.

Nucleus, centrally located and containing 1845 times the mass of any other part of the system.

Electrons (negatively charged particles) revolving about the nucleus; these are of two kinds: (Fig. 4).

(1) Lying closer to the nucleus—*nuclear*, insufficient to neutralize the + charge of the nucleus.

(2) Lying farther away *valence electrons* balancing the positive charge as a whole, of the nucleus. The number of such external electrons determines the place of the element in the periodic scale (page 32).

The nuclei of the atoms of all elements consist of either

(1) One hydrogen atom; this is the case with hydrogen itself.

(2) Of four hydrogen atoms intimately connected, not in the ordinary chemical manner, but *via* interatomic forces at present unknown; this collection of hydrogen atoms is the atom of helium, the next element in complexity. In all atoms the atomic weights of which are divisible by 4, there is but the atom or atoms of helium, but in all other elements, in addition to helium, there are hydrogen atoms. Thus, in nitrogen there are 3 helium atoms and one molecule (two atoms) of hydrogen. The atom of aluminium consists of 6 helium and 3 hydrogen atoms. Rutherford[1] is enabled to remove hydrogen atoms from these compounds by bombarding the atoms with helium atoms. These atoms are obtained in the so-called "alpha rays," generated in an electrical discharge tube. While it remains to be distinctly proven that an element of a lower order is obtained by such bombardment and loss of hydrogen, it is believed that such is the case. Aluminium has 13 external electrons which represent the number of positive charges on the nucleus; the electrons are called "valence" electrons because the qualities of the element are determined by these bodies, although they are really dependent upon the nucleus as to number, position, etc. If a hydrogen atom is knocked from an atom of aluminium, magnesium may remain and from other elements, (boron, sodium, fluorin, phosphorus),

[1] English physicist, contemporary.

corresponding elements probably arise, either as classical "elements," like beryllium, neon, etc., or an isotope.[1] This process of transmuting elements may signify that there has been an inorganic evolution in which the higher elements have been condensed from hydrogen atoms.[2]

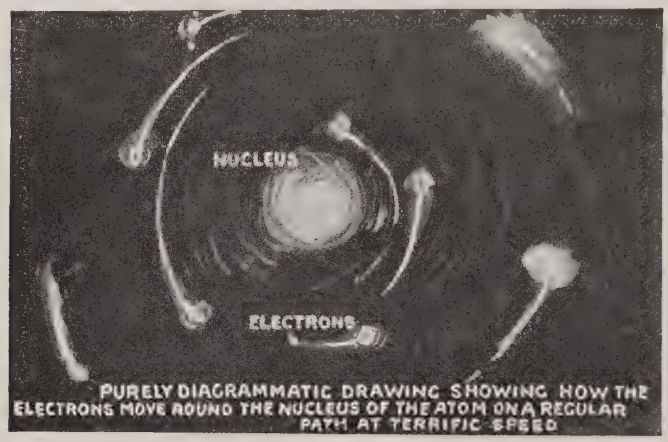

NUCLEUS
ELECTRONS
PURELY DIAGRAMMATIC DRAWING SHOWING HOW THE ELECTRONS MOVE ROUND THE NUCLEUS OF THE ATOM ON A REGULAR PATH AT TERRIFIC SPEED

Fig. 3.—After Sir William Bragg, showing the planetary relations of electrons (representing planets) moving in definite orbits around the proton (representing the sun). The mass of the atom is relegated to the proton, which is charged positively. The electrons are charged negatively. (Sir William Bragg, in Illustrated London News.)[2]

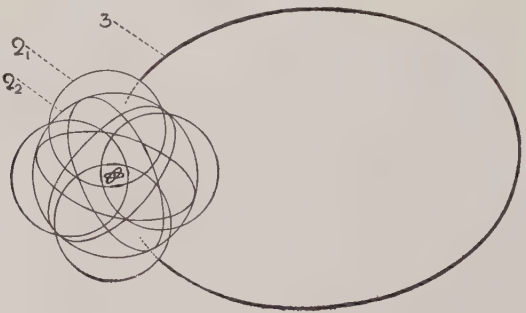


Fig. 4.—Illustrating the Bohr theory of composition of the atom. Like the heavenly bodies, the electrons in the atom move in fixed orbits. Atom of sodium having one octet (8) and three external electrons, making 11 in all. Hence the position of Na as 11 in the table on page 32.

[1] Neon, derivable from sodium (see Fig. 7) has one isotope, the atomic weight of which is 2 higher than neon, the atomic weight of neon being 20.

[2] For a simple account of the structure of the atom see articles by Sir William Bragg in the London Illustrated News, 1924, pages 676 and following. See also Bragg, William, Concerning the Nature of Things, New York, Harper, 1925. Some of the drawings in the present volume are after those in this series of articles.

Fig. 5.—Stanley R. Benedict, Professor of Biochemistry, Cornell University Medical College, New York, N. Y. Critical student and inventor of biochemical methods, contributor to the biochemistry of glucose, uric acid, creatinin, etc.

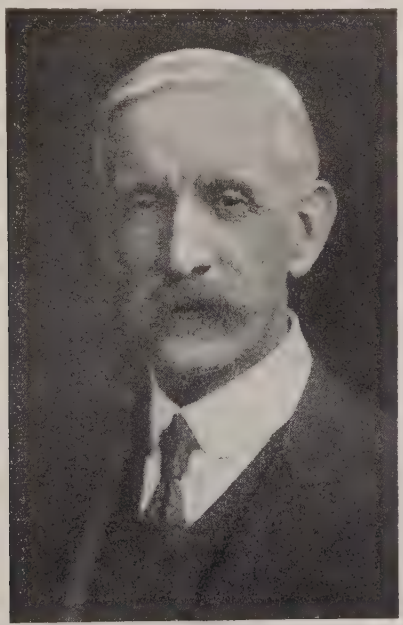
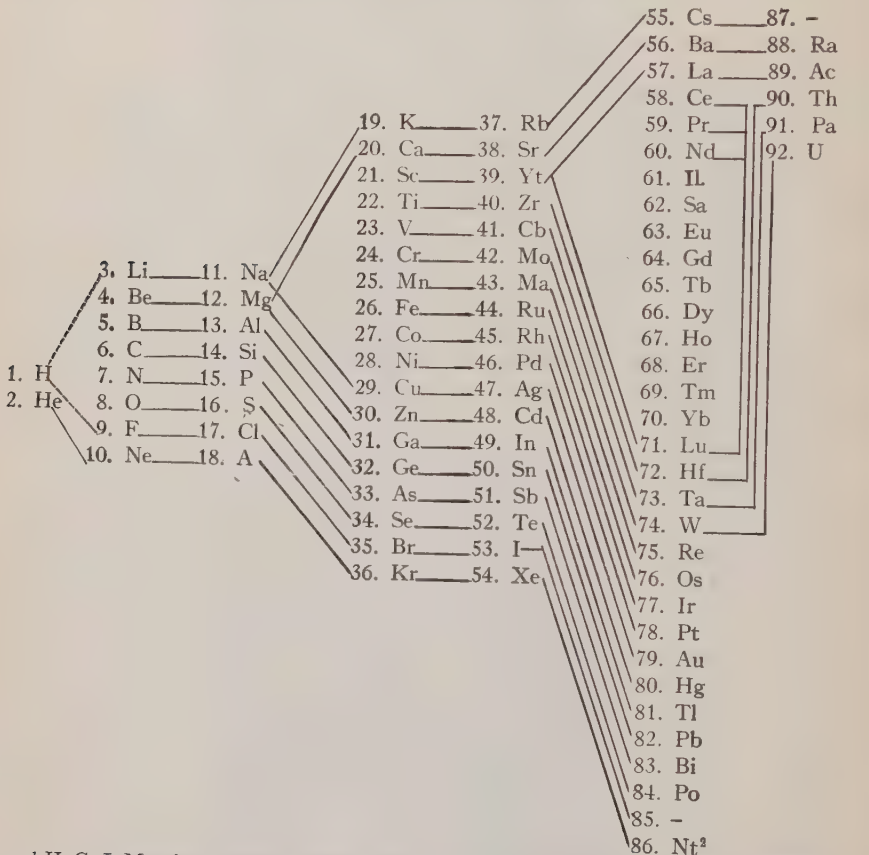


Fig. 6.—Sir F. Gowland Hopkins, Professor of Biochemistry, Cambridge University (England). Discoverer of tryptophan, glutathione, pioneer investigator of vitamins, and inspirer of many biochemical investigations.[1]

[1] Courtesy of Professor C. G. L. Wolf, Addenbrooke Hospital, Cambridge, formerly Professor of Biochemistry, Cornell University Medical College, New York, contributor to the knowledge of many biochemically important substances and processes (see Index).

TABLE OF PERIODIC ARRANGEMENTS OF THE ELEMENTS (MOSELEY)[1]

The atomic numbers are given to the left. The lines connect members of the same groups; for instance, IA of the Mendeléjeff Table (lithium, sodium, potassium, etc.) is indicated by the lines from Li to Na, etc.



[1] H. G. J. Moseley, English physicist, killed at Gallipoli, age twenty-eight. Moseley proved that the characteristic of the proton, namely, mass, increases with regular steps as we pass from one element to the next higher in the periodic scale, and that the atomic weight is not an index of the relation of the elements. The table of the atomic weights given on page 139 is from the work of Bohr.

[2] Or radon.

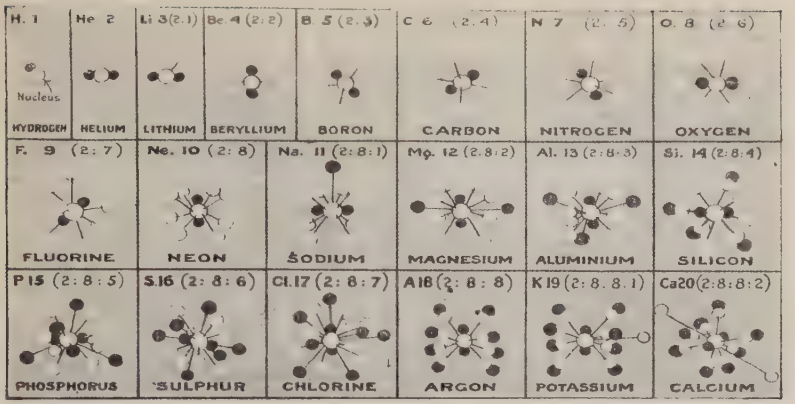


Fig. 7.—Plate, after Sir William Bragg, to illustrate the composition of the atom. The protons are shown as central spheres, the nuclear electrons as black spheres. The external (valence) electrons are grey. Hydrogen possesses a single external electron; helium, two; sodium, two central electrons, an outer octet (8) and a single outlying one. Compare Fig. 4 (Sir William Bragg, in Illustrated London News).

Fig. 7*a*.—Hydrogen atom, with one external electron. Right, helium atom with two external electrons. The orbits are shown.

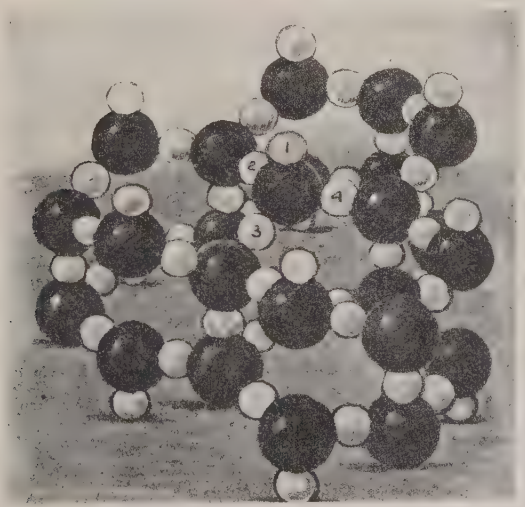


Fig. 8.—A crystal of ice showing the arrangement of oxygen and hydrogen atoms. Oxygen, large spheres; hydrogen, small. (Sir William Bragg, in Illustrated London News.)

The following diagram gives the relations of matter in different states:

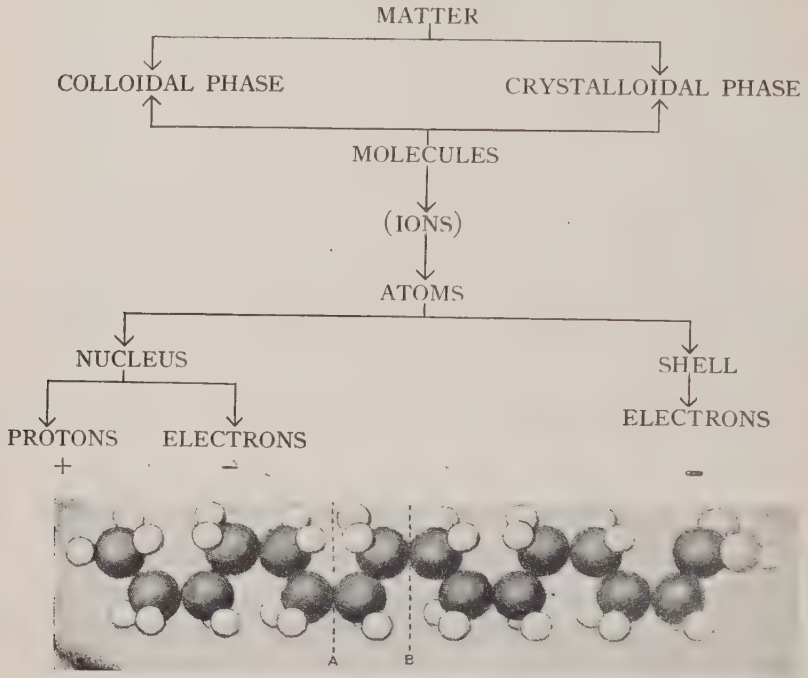


Fig. 9.—Molecule of palmitic acid. Large atoms, carbon; smaller, hydrogen, stippled, oxygen. Removing the portion of the molecule between the lines makes a molecule of myristic acid. Removing two more, makes lauric acid. (After Sir William Bragg, in Illustrated London News.)

The Union of Atoms into Molecules.—This is accomplished by one atom sharing one or more or its external (valence) electrons with the second atom. Two kinds of sharing are known to occur:

Homopolar.

The electron circulates through both atoms and does not become identified with either. The charge on each atom remains the same as before the union.

There is no tendency towards dissociation into ions.

Examples: O_2, N_2, the organic (carbon) compounds, etc.

Heteropolar.

The electron is more or less completely transfered to the second atom, thus leaving the atom whence it came more positively (less negatively) charged, while the reverse holds for the atom which it has joined.

Ionization readily occurs.

Examples: NaCl, etc.

The Chemical Atom.—According to the conception of the structure of the atom given above, usually called the "physical atom," in which the electrons are in motion, it is difficult to explain how atoms are made into molecules. In order to explain this process more easily

American chemists replace the dynamic method by a static model of the atom, and the scheme is called the "chemical atom."[1] In it it is assumed that the circulation of electrons stops in such a manner that they come to occupy the corners of a cube. The nucleus lies in

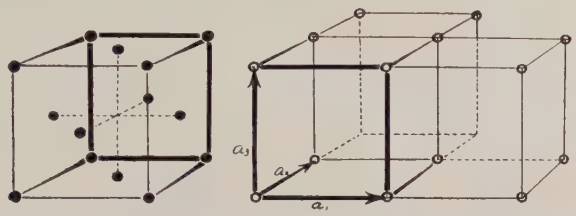
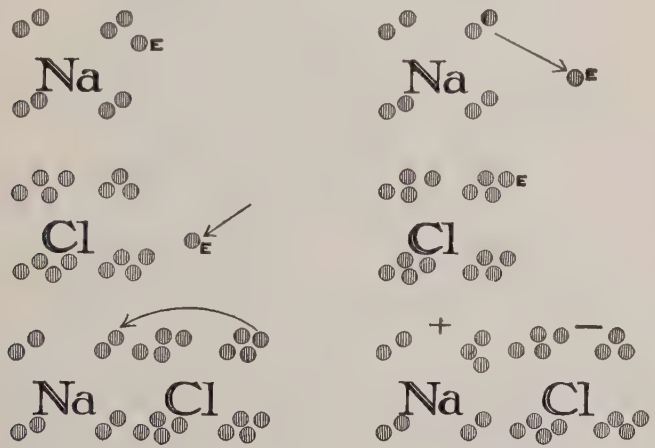


Fig. 10.—The static model of a molecule. To the right, a molecule of water, H—O—H, showing the sharing of electrons in order to combine the atoms into the molecule.

the center of the cube (Fig. 10). Such a conception may be considered as the average position assumed by the electrons in their rotations. The external electrons are present from one (H) to eight (Neon);



Ionization: In order to stabilize the atom, sodium loans an electron, leaving an octet. Chlorion stabilizes its atom by accepting an electron. When a molecule of salt, NaCl, is formed, an interchange of electrons occurs.

at this point the cube becomes "saturated," for all the corners are full. If more electrons are present, as in sodium, where there are nine

[1] G. N. Lewis, Professor of Chemistry in the University of California, Berkeley and Irving Langmuir, research chemist to the General Electric Company, Schenectady, N. Y. The basis for such models is the work of the Abbé Haeuy, who first used the "lattice structure" for crystals. Recently the younger and elder Bragg (Manchester and London) have used the *x*-ray to analyze crystal structure. The illustration (Fig. 11) is from their work.

external electrons, a new cube is started.[1] Thus, the electrons are arranged in octets, or in parts of octets (Fig. 10). When an atom unites with another atom in the formation of a molecule, duplets of two electrons are held in common, unless one atom can take on another electron from the atom pairing with it. Such transfer of electrons is known as co-valency. In the inert gases (helium,[2] neon, argon, etc.) the octets are full, and therefore there is no such process. In all other cases, when molecules are formed, there is first a rearrangement

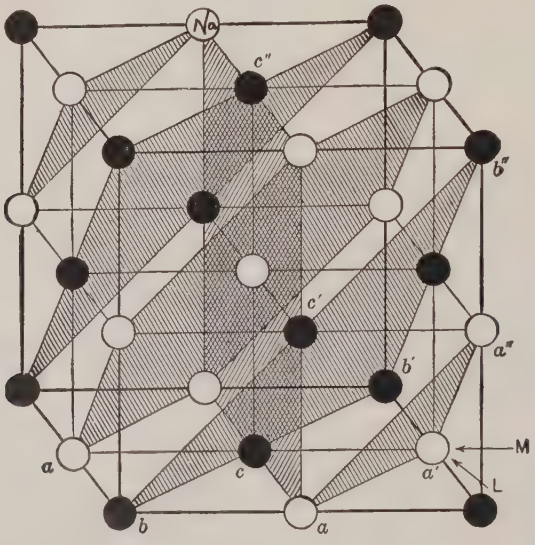


Fig. 11.—A crystal of salt analyzed by the *x*-ray method of Bragg. The sodium atoms are white; chlorin, black. (From Deming's Chemistry.)

of electrons in which they are exchanged from the outer octet of one atom with the electrons of the outer octet of the other atom. A balance is reached, leaving the atoms in stable condition, with all octets full.

Dissociation, or **ionization,** occurs when a polar substance is thrown into a solution in which "co-ordination" takes place, that is, each ion attempts to maintain its stability by losing or accepting an electron. It is necessary to believe that when NaCl dissociates in

[1] This is in conformity with the Rule of Abegg, that all atoms have the same total number of valencies, namely, eight. Thus, nitrogen in ammonia has a −3 valence, and in nitrogen pentoxid, +5, making a total range of maximum positive and negative valencies of 8.

[2] Should be called "helion," to resemble argon, etc.

H_2O that the ions H^+ and OH^- react with Na^+ and with Cl^- causing the disruption of the molecule (ionization).[1]

The Ionization of Water.—A molecule of water is a very stable substance (Fig. 12). 13.8 large Calories of heat must be applied in order that complete ionization of water occur at 20° C; this is equivalent to 66,543.6 joules of work. Water is a well-recognized insulator of electrical effects because it is a very poor conductor of the current. Kohlrausch found that water conducted the current as if it were a 1.05/10,000,000 normal solution of ions, at 25° C. Since the ion[2] carries the current or is the current, there must be few ions in pure water. Water ionizes into the cation H^+ and the anion OH^-. There are three substances in pure water: H^+, OH^-, and H_2O. The following diagram roughly represents the condition of water with respect to ionization:

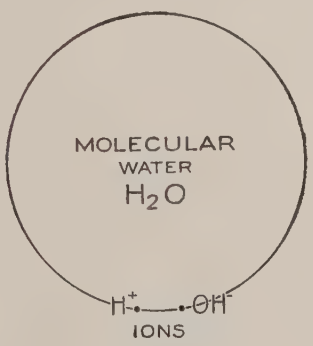


Fig. 12.—Showing relative proportions of molecule and ions in water at 20° C.

The Reaction of Fluids.—By the reaction of a fluid we mean that the fluid is acid, basic or neutral. In earlier times acidity was attributed to oxygen[3] (Lavoisier). Later it was found that oxygen does not exist in certain substances which give the acid reaction, whereas another element, hydrogen, always is present. We know now that acidity is due to the presence of hydrion (H^+) in excess of the hydroxyl-ion (OH^-). Although there is but a small amount of ionization in the purest water,[4] there is some, and by methods about to be described

[1] The theory of A. Werner (Zurich), which assumes that auxiliary valencies of the atoms differing from the valencies that hold the molecule together, react with the medium and cause dissociation of the molecule.

[2] Greek *ion*, going; that is, wandering about.

[3] See Franklin, E. C. (Ex-President of the Amer. Chem. Soc. and Professor of Chemistry, Leland Stanford, Jr. University, Palo Alto, California). Systems of acids, bases, and salts. Presidential address, Jour. Amer. Chem. Soc., vol. 46, p. 2136, 1924.

[4] The purest water is obtained by distilling through a copper distillation apparatus followed by redistillation in quartz vessels. This is called "conductivity water" because it does not conduct!

it has been found that the concentration[1] of hydrogen ions in pure water at ordinary temperatures (22° C.) is one gram-ion (1.008 g. H^+) per ten million liters of water. This is about equivalent to one gram H^+ per 85,000 barrels of water.

We may express the normality[2] of water at 24° C. by saying that it is a $\frac{1.0}{10{,}000{,}000}$ normal solution of H^+ since we are speaking in terms of liters. A mathematician would abbreviate this expression by saying:

$$\frac{1}{10{,}000{,}000} \text{ normal} = \frac{1}{(10)^7} \text{ normal} = (10)^{-7} \text{ normal.} \qquad (I)$$

Fig. 13.—Ionization of water: Eighty-five thousand barrels of water with one gram of hydrion.

Briefly stated, *the concentration*[3] *of hydrogen ions in pure water at ordinary temperature is* $(10)^{-7}$ normal. It is customary to use such an expression to designate the reaction of fluids, but in place of using the whole expression, since 10 appears in all cases, it is omitted, as well as

[1] Mass per capacity. The liter is the standard unit of capacity. A thousandth of a liter is a milliliter (ml.). It is approximately the same as the cubic centimeter, the unit of volume. A milliliter is equivalent to 1.000,027 cc. The milliliter will be used throughout this book in conformity with the recommendations of the U. S. Bureau of Standards and because pharmaceutical glassware is standardized in this system.

[2] By normality we mean the concentration compared to that in a normal solution. A normal solution is the number of grams of substance indicated by the hydrogen equivalent of the molecular weight, made up to a liter of water. By hydrogen equivalent we mean the number of hydrogen atoms replaceable with a metal. NaOH has one, since with HCl, Na replaces one hydrogen. Na_2SO_4 has two hydrogens replaceable, since the acid H_2SO_4 has two hydrogen atoms replaceable with Na. To make a normal solution of H_2SO_4, $\frac{1}{2}$ molecular weight must be taken. The normality is one-tenth (0.1 n.), one-fifth (0.2 n.), etc., normal.

[3] Abbreviated "CH."

the minus sign, leaving only the exponent,[1] in which case we no longer speak of "CH," but of *p*H. We are justified in this procedure in another way, which will be discussed later (page 61).

Definition of *p*H.—*p*H is equivalent to the expression (−log CH); it is the reciprocal or minus logarithm to the base 10 of the normality of the solution, the minus sign being omitted (but understood). It is simply a convenient way of expressing, quantitatively, the reaction of a fluid, inasmuch as the reaction is dependent upon the concentration of hydrogen ions. Two advantages are gained by its use: (1) In plotting curves of acidity of body fluids (urine, etc.) logarithms vary comparatively slightly, while their numbers have wide variations. (2) It is much easier to compare single numbers, like 1.02 than expressions such as $94.8 \times (10)^{-4}$. The logarithm is usually not a whole number, as we have indicated above. A decinormal solution of hydrogen ions has *p*H 1.0, since:

$$\text{decinormal} = \frac{1}{10}\ \text{normal} = \frac{1}{(10)^{1.000}}\ \text{normal} = (10)^{-1.000}\ \text{normal}. \qquad \text{(II)}$$

This would be the case at infinite dilution where there is complete ionization of H_2O into H^+ and OH^-. Ordinary decinormal solutions, however, do not undergo complete dissociation. A decinormal solution of HCl at 25° C. is but 81 per cent. dissociated.[2] Actually, it is a 0.081 normal solution of ions H^+ and Cl^-:

$$\begin{array}{l} 0.10 \text{ normal solution} \\ \underline{0.81 \text{ parts ionized at } 25^\circ \text{ C.}} \\ 0.081 \text{ normal solution respecting ions.} \end{array} \qquad \text{(III)}$$

This figure may be stated:

$$0.081\ \text{normal} = \frac{81}{1000}\ \text{normal} = \frac{81}{(10)^3} = 81\ (10)^{-3}. \qquad \text{(IV)}$$

This last expression is the CH. In order to express the quantity in terms of *p*H, we convert the number 81 into logarithmic terms by finding the logarithm in a table of logarithms; then we combine this logarithm with −4, remembering that to multiply logarithms, the logs are added algebraically:

$$\begin{array}{rl} & -4.0000 \\ \log 81 = & +1.9085 \\ \text{Multiplying (adding)} & -2.0915 \end{array} \qquad \text{(V)}$$

the *p*H of a decinormal solution of HCl at 25° C., the minus sign omitted.

[1] This nomenclature follows the suggestion of S. P. L. Sørensen, Copenhagen, Denmark. See Clark for picture of Sørensen and a discussion of the significance of these expressions. The reader may obtain from the U. S. Public Health Service, Washington, D. C., a pamphlet by F. H. McCrudden (Surgeon), giving a statement of these factors. (Reprint No. 300, February 17, 1922.)

[2] Activity coefficients are used; see page 40.

The Neutral Point.—We have stated above (page 38) that the CH of pure water is practically $(10)^{-7.0}$ normal.[1] The *p*H is then 7.0. Ionization varies with the temperature, and this figure represents the *p*H of water at a definite temperature, namely, 22° C. At temperatures below and above 22° C. the *p*H is different:

16° C.	*p*H 7.10
18° C.	*p*H 7.07
20° C.	*p*H 7.03
22° C.	*p*H 7.00
24° C.	*p*H 6.96
26° C.	*p*H 6.93
28° C.	*p*H 6.90
37° C. (body temperature)	*p*H 6.75

Water is tasteless. It is neutral because it dissociates into the same number of hydrogen ions and hydroxyl ions.

Concentration and Activity.—In our discussion, we have used the term contration as if the chemical activity of substances in solution depended upon the amounts. This is true for substances, like hydrogen in water, which dissociate feebly. In complex mixtures of substances and in concentrated solutions, chemical activity does not proceed exactly according to the concentration. This fact was recognized by the founders of the Law of Mass Action, Guldberg and Waage, who spoke of *active mass* rather than concentration. Their Law is usually expressed as follows:

$$\frac{\text{Active Mass of Cation times A.M. of Anion}}{\text{Undissociated molecular mass}} = \text{a constant.} \qquad \text{(VI)}$$

The *p*H Scale.—The *p*H of a normal acid solution is 0.00, because the log of 1 is zero; *p*H = 0.00.

The *p*H of a normal base solution is 14, because

$$(H^+) \times (OH^-) = 14 \text{ (Cf. Eq. IX, page 41).}$$

and since $-\log (H^+) + -\log (OH^-) = 14$, and $-\log (H^+) = 0$, $-\log (OH^-)$ must be 14 to satisfy the equation.

Again a decinormal solution of a completely dissociated substance has *p*H = 1.0 (Eq. II, page 39). A decinormal solution of base is *p*H = 14 − 1 = 13. The lower the *p*H number, the more acid the solution and the higher the *p*H number, the more alkaline: We may erect, then, a line to represent this range:

*p*H 1.0	5.0	7.0	7.3	8.0	13.0
I	I	I	I	I	I
Decinormal acid.	Urine.	Neutral.	Blood.	Maximum intestinal reaction.	Decinormal alkali.

[1] Practically, because there may be a secondary dissociation in which the hydroxyl ionizes: $H_2O = H^+ + OH^- = 2H^+ + O =$. The most exact determinations give *p*H = 7.07 at 18° C. See page 41, note 4.

The Ionization Constant.—The chemically active substances in water are the ions H^+ and OH^-. When they react they produce molecular water, H_2O. The Law of Mass Action expresses the relation between the active mass and the product. For water this is:

$$(H^+) \times (OH^-) = (H_2O) \times k_a \qquad \text{(VII)}$$

where the brackets indicate active mass and k a constant varying with the kind of substance under consideration.[1] From this expression we find:

$$\frac{(H^+) \times (OH^-)}{(H_2O)} = k_a \qquad \text{(VIII)}$$

but we have seen (Fig. 12) that there is an almost infinite amount un-ionized water compared to the amount of ions and hence we may disregard molecular water in the above equation; then we have:

$$(H^+) \times (OH^-) \overline{\overline{\times}} K_w^2 \qquad \text{(IX)}$$

The quantity K_w is called the ionization constant. The expression signifies that no matter how many hydrions there are, there must be hydroxylions sufficient to make the equation balance. We have seen above that at neutrality

$$pH = pOH = 7.0. \qquad \text{(X)}$$

Substituting this figure in the above equation, we have

$$7 + 7 = 14^3 \qquad \text{(XI)}$$

This is the ionization constant[4] for water at 22° C.

Calculations of *p*H.—Rule to find the *p*H of a solution, given the CH:

(I) Convert the common fraction into decimal fraction, the denominator being expressed as power of 10. Ex.: $\frac{14.2}{100{,}000}$ normal $= 14.2\ (10)^{-5}$ normal.

(II) Add, algebraically, the log of the number standing before 10, to the exponent of 10. Ex.: Log of 14.2 is $+ 1.152$
$- 5.000$ (XII)
Combined log, $- 3.848$

(III) Express *p*H by omitting the minus sign. Ex.: *p*H = 3.848.

[1] In this case H^+ and therefore acid (a).

[2] A new constant is used in this case, where w refers to water.

[3] Because *p*H = (−log CH) and logs are added when numbers are multiplied.

[4] This constant has been determined by many observers, and slight differences are obtained according to the method used. The Dutch chemist, Wijs, found $1.44(10)^{-14}$; the American chemist, G. N. Lewis, found $1.006(10)^{-14}$. Lewis' figure corresponds to *p*H 6.9987.

Rule to pass from *p*H to CH:

(1) From the integer[1] next above the *p*H figure, subtract the *p*H. Ex.:

*p*H 3.848. The next whole number is 4.000

Subtracting ..... 3.848 (XIII)

0.152

(2) Find the antilog[2] in a book of logarithms. Ex.: Antilog of 0.152 is 1.42.

(3) Write the expression for CH by placing the number just found before 10, the exponent of 10 being the integer in (1), with the minus sign. Ex.: The number found in (2) is 1.42.

The integer found in (1) is 4.000.

Then CH is $1.42\ (10)^{-4}$, which is the same as $14.2\ (10)^{-5}$.

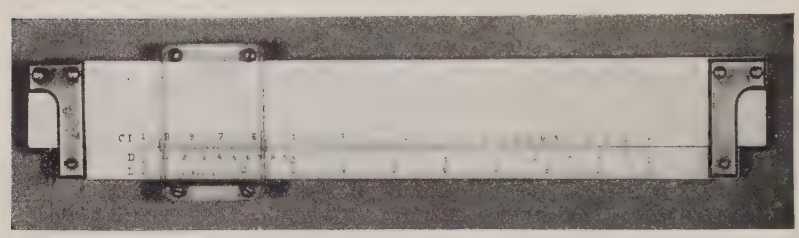


Fig. 14.—A Duplex slide-rule, with inverted scale: C1 useful in converting *p*H into CH, and vice versa. L is the common logs of numbers on the D scale. For directions see text, below. As the rule is set, scale L reads the fraction to right of the decimal point in the *p*H, namely, 0.14, and the scale CI reads the number preceding the expression "$(10)^{-}$" in the CH; in the above setting it is 172, the decimal point being determined by the particular case.

Calculation by Duplex Slide-rule.—The duplex slide-rule[3] (Fig. 14) affords a ready means of making these calculations. The scale of equal parts (L) gives the fraction to the right of the decimal point in the *p*H expression (the figure 848 in *p*H 3.848) and the scale CI (inverted) gives the number that stands to the left of $(10)^{-n}$ in a CH expression (14.2 in the expression $14.2(10)^{-5}$). The negative exponent, -4, is always one greater than the whole number (3) of the *p*H. The antilog and logs are readily found on these rules and they are quite convenient.

Strong and Weak Acids.—In hospital work boric acid[4] is used as a weak antiseptic. It is one of the weakest acids. It ionizes to an extent less than 0.01 per cent. in decinormal solution, as compared with hydrochloric at 81 per cent. The following table gives the percentage ionization of some common acids:

[1] Whole number.

[2] The antilog is the number in the log-book corresponding to the log.

[3] Or an ordinary rule with the slide reversed. A duplex rule is shown in Fig. 14.

[4] Frequently called "boracic acid," a safer term than the curt "boric" in the sick-room, but chemically improper.

ACTIVITY COEFFICIENTS OF SOME COMMON ACIDS, BASES AND SALTS
25° C.

Acids		
HCl	0.814	0.10 normal
H_2SO_4	.313	0.10 "
"	.617	0.01 "
Salts		
NaCl	0.798	0.1 N.
KCl	.794	"
KNO_3	.732	"
$BaCl_2$	.501	"
"	.716	.01 N.
K_2SO_4	.421	0.1 N
Bases		
KOH	0.800	

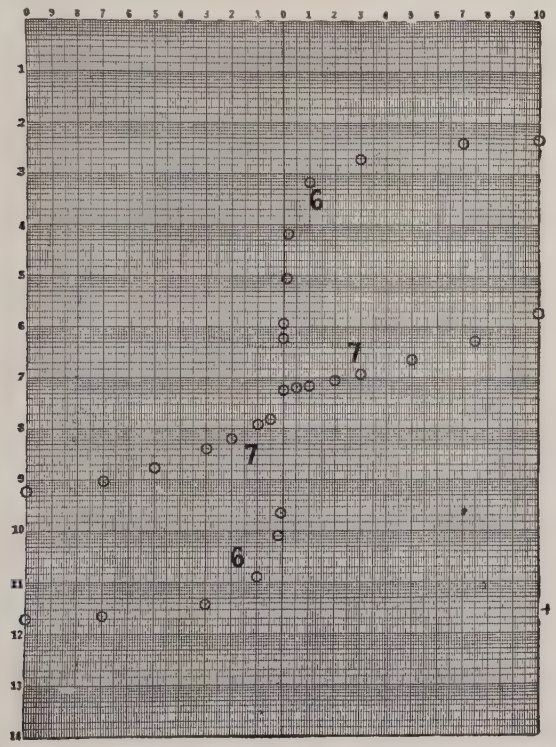


Fig. 15.—Titration curves: Curve 6 to right, HCl added to distilled water; *p*H from 7 to 2.4. Curve 6 to the left, NaOH added to water; *p*H from 7 to 11.8. Curve 7 shows the buffering effect of gelatin; the additions are the same as in Curve 6, but there is much less change in *p*H. Courtesy A. H. T. Co. after graph made by the Bovie direct reading potentiometer.

EXERCISE 1.—*Strong and Weak Acids.*—Obtain from the storeroom 25 mls. of each of the following solutions (all decinomal): (I) HCl; (II) CH_3COOH; (III) H_3BO_3. Clean the end of a stirring rod

and transfer one drop of (I) to the tip of the tongue.[1] Immediately after detecting the taste, rinse the mouth with water and proceed with (II) in the same manner. Continue until the whole series has been sampled. Record in your note-book your experience in each case and correlate your findings with the table given above (page 43).

Exercise 2. Secure from the store-room 10 mls. each of a half-molecular solution of table sugar, sucrose. Place 3 clean test-tubes in your rack and label them A, B, and C. Add to:

A, 1 ml. sugar solution and also 5 mls. of 0.01 normal HCl.[2]
B, 1 ml. sugar solution and also 15 mls. of 0.01 normal acetic acid solution.
C, 1 ml. sugar solution and also 10 mls. of distilled water (control).[3]

Place the 3 tubes in a beaker of boiling water, or a boiling water-bath for exactly 5 minutes. Cool. Dilute to 25 mls. with distilled water. Transfer the contents of A (25 mls.) to a burette. Mix the contents of the burette by stoppering the open end with a stopper and inverting the tube several times. Now transfer to an evaporating dish exactly 25 mls. of Benedict's solution[4] for the quantitative determination of reducing sugar, by means of a 25 ml. pipette. Add about 5 gs. desiccated sodium carbonate. Place the evaporating dish under the burette, on a tripod over a Bunsen burner and bring the contents to a gentle boil. Add the sugar solution from the burette, drop by drop, continuing boil, until the greenish-blue of the cupric sulphate is discharged, leaving a colorless liquid. Read the burette and enter the reading in your note-book. Repeat this procedure with each of the solutions B and C. Twenty-five mls. of Benedict's solution oxidize 0.050 g. of sugar as glucose. Calculate for each solution the amount of glucose obtained from the sucrose by boiling with acid. Which acid causes the largest amount of glucose to be produced in one minute?

Quantitative Determination of the Number of Ions in Solution. We have said that ions carry electricity and that the conductivity of a solution depends upon the number of ions present. These are substances with definite properties; the hydrion, for instance, causes acidity. Hydrion is a definite substance, as hydrochloric acid is a definite substance. Ions react with other substances. In a solution, each particle attracts other particles, and for this reason a solution

[1] The taste-buds responding to acid lie on the tip and sides of the tongue.

[2] By properly diluting the decinormal HCl of Exercise 1.

[3] Every experiment must have a control or controls. In the above experiment we are adding water in each case along with the special substance, like sugar, HCl, etc. What effect has water itself? Many scientific errors have been the result of omitting the control.

[4] Page 160 and Appendix.

containing an ionizable substance does not boil as readily as one containing the same amount of substance which does not undergo dissociation. In the first case, where substances are ionized, there are more particles to react upon one another, and these bodies keep the water molecules from bounding above the surface of the liquid, which is the phenomenon we know as boiling. On the other hand, the greater the number of ions in a solution, the lower the freezing-point.[1] Freezing involves an orientation or "disciplining" of molecules, and the rapidly moving ions like Na^+ and Cl^- do not lend themselves to discipline. We have, then, the following methods for determining the degree of ionization in solutions:

(1) Electrical conductivity.

(2) Elevation of the boiling-point.

(3) Depression of the freezing-point.

The data given by the three methods are not identical owing to inter-ionic attractions which become more evident the stronger the solutions. The nature of these inter-ionic attractions is discussed by Noyes.[2]

(1) **Conductivity.**—The divided current principle of the Wheatstone bridge is employed. The following diagram (Fig. 16) illustrates the principle:

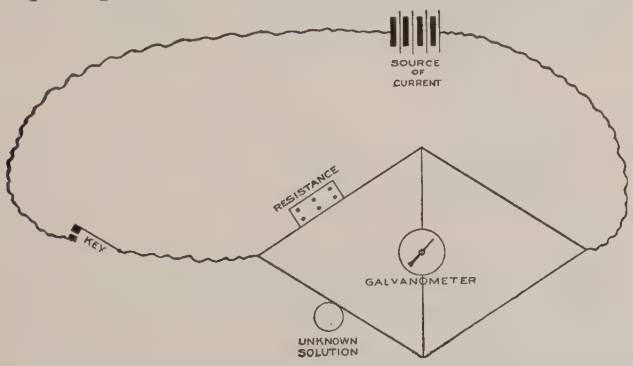


Fig. 16.—Principle of the determination of conductivity of solutions. The current, arising from the source above, divided exactly equally into the two arms, one of which bears the resistance box and the other the cup for the unknown. By means of the "null-point" instrument (galvanometer) connecting the two arms it is possible to tell when the current in one arm exactly equals that in the other; this is brought about by changing the resistance in the box. In equilibrium the conductance through the unknown is the reciprocal of the resistance in the box.

[1] Common salt is thrown upon icy pavements in order that the ice melt. Salt solution freezes at a lower temperature than pure water, and at 0° Centigrade the solution remains unfrozen, although water freezes at that temperature.

[2] Jour. Amer. Chem. Soc., vol. 46, page 1080, 1924 in the light of the highly mathematical developement of two European physical chemists, Debye and Hückel.

The solution contains ions and molecules and electricity is transferred in proportion to the number of ions. The current is derived from the battery ("source of current"). By introducing resistance (resistance box, Figs. 16 and 17) into the upper arm of the apparatus the point is reached at which the galvanometer shows no more deflection when the key is closed. Resistance is introduced by withdrawing the

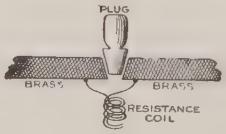


Fig. 17.—Details of the conductivity method, one of the units in the resistance box; when resistance is desired the plug is removed in order that the current pass through the coiled wire, the exact resistance of which in ohms is known.

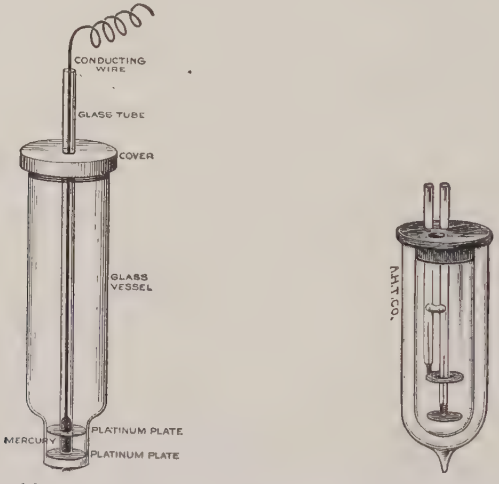


Fig. 18.—Ostwald type conductivity vessels. The conductivity of the fluid lying between the upper and lower platinum electrode plates is determined. For critical work, the improved vessel shown on the right is used. The electrodes are contained in a Dewar flask ("thermos bottle") which inhibits gain or loss of heat. The position of the vessel in the apparatus is shown in Fig. 19.

plugs, which, as long as they are in place, permit the current to pass through the brass with practically no resistance, but which, when withdrawn, cause the current to pass through the fine wire connecting the sides of the brass plates (Fig. 17). These wires are calibrated in terms of conductance which is the reciprocal of resistance:

$$R = \frac{1}{C}$$

The unit of conductance is the mho, the reverse of the word for the unit of resistance, the ohm.

Demonstration. *The Conductivity of Decinormal Solution of Hydrochloric Acid.*—This is done by using the Wheatstone bridge, galvanometer, cell, resistance, and Ostwald conductivity vessel (Fig. 18). The meter bridge consists of a meter stick with terminals for wires at each end connected with the bridge-wire, which is a fine wire running from one end of the stick to the other. A contact is provided which may be slipped along the wire. The meter-stick

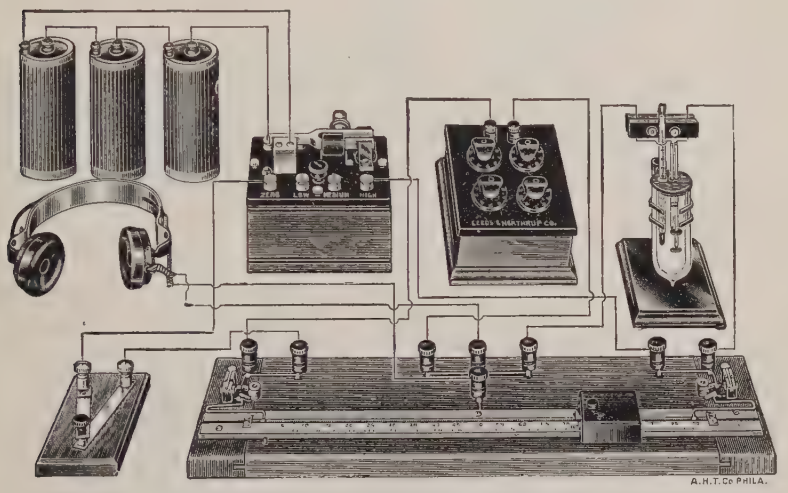


Fig. 19.—Conductivity apparatus. The resistance $\left(\frac{1}{C}\right)$ of the unknown placed in the vessel (right) to a current activated by the batteries (left) is estimated by means of the resistances of the box (upper right) and the meter-bridge (front). The "no-point" is determined by the alternating current set up by the inductorium (upper left); the telephone receiver (left) detects variations in the current.

with its wire supplements the coarser resistance of the box. Since the wire is stretched along a meter-stick, it is divided thereby into thousandths (millimeters) and hence the readings are in milliohms. In place of a galvanometer as "null-point" instrument, a telephone (Fig. 19) may be introduced, the current for which being either a small inductorium, or the alternating[1] house-current suitably cut down. When there is no sound in the telephone, or when the galvanometer is undeflected, the current from the resistance box and slide-wire exactly balances that from the conductivity vessel containing the solution of decinormal hydrochloric acid. The quantity R is obtained by counting up the ohms represented

[1] Not the direct current.

by the plugs removed and also the position of the slide-wire contact, on the meter-stick. Then

$$C = \frac{1}{R} \qquad \text{(XIV)}$$

After this result has been obtained, the conductivity cup is washed out and the procedure is repeated with decinormal acetic acid.

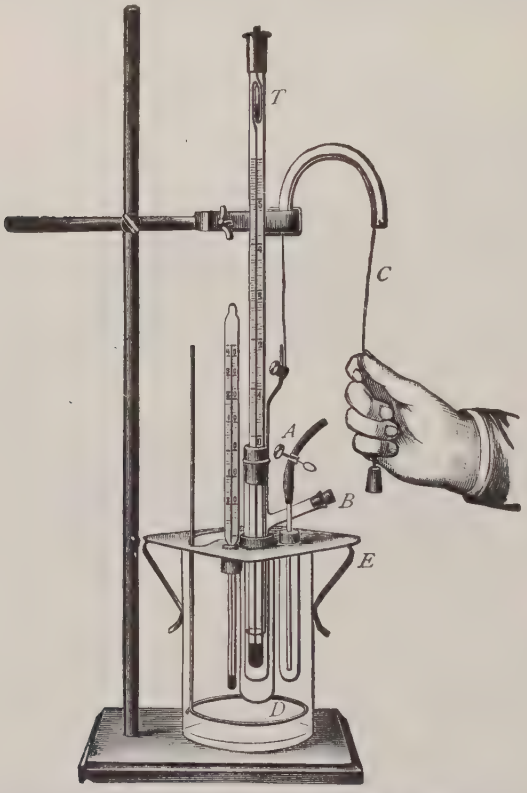


Fig. 20.—Cryoscopic apparatus for determining the difference in freezing-points of a pure solvent like water and with a solute like the constituents of blood. A battery-jar containing a wire stirrer (*D*) is filled with a salt-ice mixture. Suspended in this mixture is a small thermometer to avoid supercooling; also a large test-tube bearing within it a smaller, side-neck tube, *B*. In this tube the unknown and its solvent are placed. A delicate thermometer (*T*) is in direct contact with these fluids. (See Fig. 21.) A hand-operated stirrer is shown at *C*. In order to save time the unknown fluid is previously brought to freezing in the tube *A*. (From Holland, Medical Chemistry and Toxicology.)

(2) **Cryoscopy**[1] (or the method of the determination of ionization by freezing-point estimation).—*Method of Beckmann.*[2]—Principle (Fig. 20). To determine Δ,[3] or the depression of the freezing-

[1] Greek *cryos*, ice, and *skopis*, sight.

[2] German chemist. The principle is that of F. M. Raoult, French chemist, 1884.

[3] The Greek letter delta.

point, the temperature at which the pure solvent, like water, freezes is first determined by means of a very delicate thermometer, and then the determination is repeated after introducing a known amount of the substance being studied.

Procedure: A Beckmann differential thermometer[1] is generally used. This is a thermometer (Fig. 21) about 60 centimeters long with about 6 degrees of scale accurately divided into hundredths of degree Centigrade. In order to facilitate the reading, a reservoir for mercury is provided at the top of the instrument as well as at the bottom (Fig. 21). By means of this reservoir it is possible to so adjust the length of the column of mercury that it appears on the scale when freezing has occurred. If the temperature is elevated, the column does not remain on the scale, in which case one must warm the mercury by means of the hand and cause it to siphon into the upper reservoir. Then the column is broken by a slight jar with the finger, so that the column, when freezing occurs, remains on the scale. When the freezing-point is reached while the column of mercury is below the scale, the column is siphoned as before into the reservoir at the top of the tube and more mercury is attached. The thermometer is slipped into a stopper which is fitted into a test-tube with side neck (Fig. 20). In order to insulate this tube, which contains the substance under examination, so that heat will neither be gained nor lost, the side neck of the tube is slipped into a stopper which fits into a larger test-tube, the latter remaining empty. The tube bearing the smaller, side-neck tube and thermometer is then inserted into a freezing mixture, the temperature of which must not be too far below that of the freezing-point of the solvent.[2] When ready, the freezing mixture is stirred gently and the column of mercury will be seen to siphon and to move down the thermometer. This will continue for a time; then suddenly the mercury will rise to a certain point and remain stationary. This is the freezing-point of the solvent. Now, through the side neck of the inner tube, a weighed amount of solute, or substance under examination (concentrated HCl, for example), is added to the solvent; or one may replace the solvent with the unknown solution itself. The difference in freezing-points is delta, the quantity sought. The reader is warned to distinguish between the freezing-point of a solution and Δ, the

[1] Differential, because it does not register the temperature over more than six degrees Centigrade. It is used as a comparison thermometer to tell the difference between freezing temperatures.

[2] Because supercooling results, which interferes with rapid and accurate work. Supercooled liquids are such substances as glass.

difference in freezing-points of two solutions, one being the solvent. The Δ of urine depends upon such constituents as NaCl, dissolved in H_2O (pure solvent).

DEMONSTRATION.—Demonstrate the difference between the Δ of decinormal HCl and of 0.1 normal acetic acid. Compare the figures with those obtained for differences in conductivity determined by the method in (1). Consult page 121, dealing with interionic attractions.

Calculations for Concentration, Etc.—The following expression gives the relation between mass of solvent, of solute and molecular weight of solute:

$$\text{Gms. solute} = \frac{\text{Mol. wgt. of solute} \times \text{wgt. solvent}}{1.86}\Delta,$$

if water is the solvent and wgt. solvent is expressed in kilos. (e.g., 125 gms. = 0.125 kilo).

The *determination of the boiling-point* is done with similar apparatus, but for practical purposes the freezing-point, which is more easily determined, will usually give the necessary information.

Method of Determining the Concentration of Individual Ions.—If we are dealing with a solution of a single substance, like acetic acid, we may determine the concentration of ions by the conductivity method; indeed by this method Kohlrausch, the German physicist computed the dissociation constant (page 41) of water. If, however, our problem is to determine the concentration of anyone kind of ion, in a mixture of ions, such as Na^+, Ca^{++}, H^+, etc., we must utilize another method.[1] In biochemistry it is seldom that a solution consists of one kind of substance. Blood, urine, gastric juice, and spinal fluid have many kinds of ions. If we desired to determine the reaction of these fluids we must obtain the concentration of hydrogen ions, or, in other words, their *p*H, because reaction depends upon the equilibrium expressed by the equation already given:

$$(H^+) \times (OH^-) = K_w \qquad \text{(XV)}$$

Fig. 21.—Beckmann type thermometer used in calorimetry, cryoscopy, and other biochemical processes. The scale covers only about 6° C. and is divided into 0.01°. The reservoir at the top permits adjustment of the column of mercury for different freezing-points.

[1] Page 52.

If there is a larger number of H^+, the reaction is acid, and an excess of OH^- ions (or fewer H^+) produces an alkaline reaction.

The principle upon which the determination of the concentration of individual ions rests is the same for all types of ions. The disease rickets,[1] which affects many children and some adults, is due to an imperfect balance of calcium in the body; it is therefore desirable to know the degree of concentration of the calcion in the body, normally and pathologically. The sodium ion participates in the regulation of neutrality in the organism,[2] while at the present time the hydrion holds the greatest interest owing to its relation to the reaction of the body fluids.

The principle by which the concentration of an ion is obtained in the presence of other ions is as follows: We have shown[3] that charged

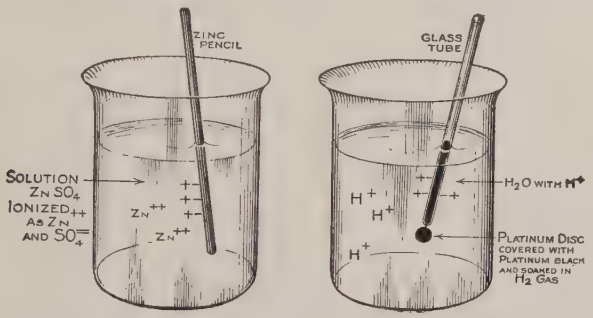


Fig. 22.—Diagrams to explain the hydrogen electrode. The potentiometer determines the potential difference between an electrode, like zinc or hydrogen, and a solution of known concentration of ions (Zn, H, etc.).

particles tend to leave substances and to wander or diffuse as ions through the medium surrounding the substance from which they come. If the medium around a stick of zinc is water, the ions Zn^{++} diffuse through the water, leaving the zinc with a double layer of charges, known as the Helmholtz double layer (Fig. 22).[4] Eventually, some of the ions migrate back towards the stick of zinc, or electrode and set up a potential difference. If the electrode is placed, not in water, but in a solution of zinc ions, more ions will migrate back to the electrode which becomes more positive the higher the concentration of ions in solution. The potential difference is proportional to the concentration of zinc ions.

[1] Chapter XIV.

[2] Page 84.

[3] Page 35.

[4] The thickness of this layer is dependent upon the concentration, but is about the size of the diameter of the ions.

The Hydrogen Electrode.— We are not interested at present in zinc ions, but the concentration of hydrogen ions must be determined in all studies of the reaction of body fluids, such as urine, blood, and gastric juice. We cannot use a stick of hydrogen because this element is gaseous at the temperatures in which we work. We may make a hydrogen electrode, however, by taking advantage of the power[1] of certain metals to absorb hydrogen. Platinum is such a substance; palladium is another and one which absorbs hydrogen to a greater extent. The power of absorption of hydrogen is greater if the surface of the metal exposed to hydrogen is increased. To this end we cause finely divided platinum to be deposited upon a platinum disk (Fig. 22) by electrolysis of a platinic chlorid solution. Then we bring this platinum-coated disk into contact with pure hydrogen gas and the colloidal[2] platinum "adsorbs," or soaks up some of the hydrogen gas, thus forming a hydrogen electrode (Figs. 22 and 26) which is used just as if it were a stick of hydrogen endowed with negative charges. The hydrogen electrode is placed in a vessel containing acid, that is, hydrogen ions, and the same effect is obtained as in the case of zinc: Hydrogen ions leave the electrode and migrate out into the solution thereby creating a difference in potential. Here, as before, the potential difference is between the hydrogen ions in the solution and the hydrogen on the electrode and is proportionate to the number of ions of hydrogen in the solution.

[1] This power is derived from the fact that when gas molecules (like hydrogen) strike a metal (like platinum, palladium or gold) they become dissociated into atoms which accept electrons from the metal and become stabilized (page 35). However, the condition is above the dissociation pressure and they dissociate again (farther on in the metal) they accept an electron and become stabilized. In this way the gas is "absorbed" by the metal. If a current of electricity is passed through the electrode, a certain interference is encountered which varies inversely with the power of the metal to pass the gas atoms. An extra potential is necessary to overcome this interference. This extra potential is "overvoltage." The following table gives the overvoltage of same metals for hydrogen; it is readily seen why hydrogen electrodes are made of platinum, for these have the least overvoltage.

Platinum (platinized)	0.005 volt
Gold	.170
Nickel	.140
Iron	.170
Lead	.360

For an account of diffusion of gas through metals see Johnson, F. M. G. and Larose, P., Jour. Amer. Chem. Soc. vol. 49, page 312, 1927.

[2] Page 100. The process of adsorption is undoubtedly a chemical phenomenon and not a simple physical process.

Therefore, to determine how many hydrogen ions there are in the solution, it is only necessary to devise some means of measuring the potential difference.

The Measurement of Potential Difference.—The measurement of electrical currents utilizes two factors; the first of these which is expressed in volts is the intensity factor or the difference in potential. The second which is expressed in amperes is the capacity factor, or the rate of current flow. We may use as an analogy two reservoirs of water connected at the bottom by pipes of different cross sections closed by valves.

The level of the water in the two systems determines whether or not there will be any flow when the valves are opened. If the level is higher in one than in the other, *i.e.*, there is a difference of potential established, then the water will flow from the system of higher potential to that of lower. The rate at which the flow occurs will be governed by the size of the valve that is opened, *i.e.*, the resistance which the pressure of the reservoir has to overcome. When the level of water is the same in both systems, no flow will occur.

In an electrical system the fall of Potential V divided by the resistance, R, equals the Current I.

$$I = \frac{V}{R}$$

We can make use of this fact in determining the potential difference by measuring the current produced. This is accomplished by utilizing the method of Poggendorf which consists of opposing the current coming from a known source by that produced by the fall in potential in the system to be measured, until we have neutralized it. It is necessary that the known source have a greater voltage than the unknown. The difference of potential is applied to a long wire of uniform resistance so that the fall of potential along the wire varies directly as the length. The unknown is connected so that the side of higher potential is connected to the side of higher potential in the known system. The other terminal is moved along the wire until the indicator shows that no current is passing.

The Potentiometer.—This is an instrument for measuring the fall of potential between two electrodes. It is virtually a Wheatstone Bridge with a resistance box for adjusting a known electric pressure against the current coming from the hydrogen electrode. It includes a galvanometer, or null point instrument,[1] to show when the two currents are equal; at this time the needle will show no deflection.

[1] A capillary electrometer can be used. The potentiometer may require a galvanometer in addition to the potentiometer as in the instrument described below.

The Source of the Known Current.—This is a cell of known strength, expressed in volts. The cell usually employed has a value which is due to the fall of potential between the elements mercury and cadmium,[1] and when made up as the Weston type[2] is made with pure cadmium sulphate, mercuric sulphate, mercury, and cadmium element, the voltage will be slightly over a volt, 1.0183 volt when measured at 20° C.[3]

The Use of the Known Current of the Standard Cell.—In continued use a standard cell would soon run down and it would no longer be standard. In order to avoid this difficulty it is customary to standardize a dry-cell or storage-battery current against the standard cell and to use the battery potential for the potentiometer. Comparisons with the standard cell must be made at frequent intervals.

The Hydrogen Electrode Current.—No practical means has been discovered for determining the potential of a single hydrogen electrode such as we have described (Fig. 22, page 51). One might use two hydrogen electrodes, one dipping into a known hydrogen solution,[4] but the difficulty is that the introduction of an unknown solution would require that we assume no change in the electrode after it has been standardized, which would probably never be the case. In order to make the system more constant we use the current developed from the fall of potential between mercury and platinum in the so-called mercurous chlorid or calomel electrode. As in the case of the cadmium cell, where, by making up the cell in a definite manner, the value of the current becomes known, so, likewise, if the calomel element is made up in a certain way[5] the potential difference will be

[1] Cadmium is used because its salts, like the sulphate used in these methods, are less readily hydrolyzed than other metals, like zinc. Cadmium stands about half-way in the electrochemical series. (See Deming, H. G., General Chemistry, New York, John Wiley & Son, 1924, p. 384.)

[2] Weston, E. Anglo-American electrician, founder of the Weston Electric Instrument Co., Newark, N. J.

[3] This simply means that in making up the cadmium cell, of Weston type, cadmium sulphate is saturated in aqueous solution at 20° C. It is known as a saturated cell. If the solution is made saturated at 40° C., or near the freezing-point of water, it does not matter what temperature is used in the determination in which the cell is used. It is necessary that each Weston cell be labeled as to its being of one or the other type, and also its voltage, determined by comparison with an absolutely standard cell such as those maintained at the Bureau of Standards, Washington, D. C.

[4] A .2 normal H_2SO_4 solution contains about a normal concentration of H^+.

[5] Accomplished by covering a layer of pure mercury in an electrode vessel (Fig. 27) with calomel and over the calomel a layer of saturated, normal, or decinormal potassium chlorid solution, depending on the type of the cell, is placed. If the KCl is saturated, then a current of electricity flowing from a cell having for one electrode this

constant and known. Hence we have the requisites for a cell which develops a current, namely, two electrodes, the one being the calomel electrode and the other the hydrogen electrode. Since we know the potential difference between the platinum and mercury in the calomel half-cell, the unknown is the potential difference between the hydrogen electrode and the unknown solution of hydrogen ions (blood, urine, etc.). Obviously this potential difference in the calomel element will add to the total current produced by the hydrogen cell, and hence we must subtract the value of this potential difference from the observed potential difference obtained by the use of the potentiometer.

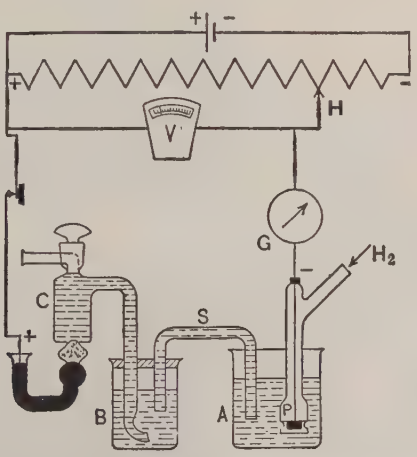


Fig. 23.—The voltmeter-potentiometer method for determining *p*H. A voltmeter V (calibrated potentiometer), reads the difference in potential between the cell composed of the hydrogen (lower right) and calomel (lower left) elements, and the cell shown in middle, top, the voltage being regulated by the resistance shown immediately above the voltmeter. (Deming's Chemistry.)

Demonstration of the Method of Determining Hydrogen-ion Concentration of Normal Blood.—(1) *By Means of the Voltmeter.*—The arrangement of the apparatus is shown in the diagram (Fig. 23). The blood is placed in the hydrogen electrode vessel A, and the electrode, saturated with hydrogen gas,[1] is lowered into it. Communication is made with the other electrode of the cell, the calomel electrode, C, by means of the liquid junction, B and S, consisting of a tube with

element and for the other a hydrogen electrode immersed in a normal solution of hydrogen ions at 20° C., has an E. M. F. of 0.248 volt. If a normal solution of KCl is used in making the element, then the potential difference betwen calomel and normal hydrogen solution is 0.283 volt. Finally, a decinormal KCl electrode gives 0.3377 volt.

[1] The arrow shows the mode of entrance of the gas.

KCl[1] of the same strength used in making up the calomel electrode. The cell, A, B, and C, has a current which is balanced against that from the battery (top).[2] The rheostat R is regulated by means of the sliding contact H. When the two currents are equal, as shown by the stationary position of the mercury in the capillary electrometer, the voltmeter reads directly the potential difference. We shall defer the calculation until after the consideration of the second method.

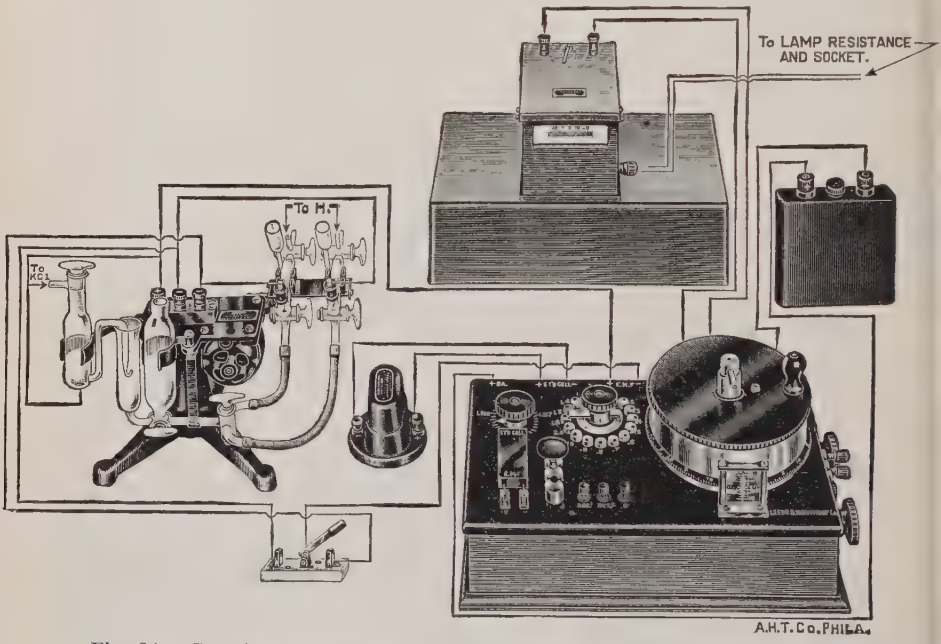


Fig. 24.—Complete apparatus for the electrometric method of determining *p*H From left to right: Stand bearing oscillating clamps holding calomel and hydrogen electrode vessels (compare Fig. 27); key for switching circuit from one H^+ electrode vessel to the other; standard cadmium element; (above) galvanometer; (below) potentiometer; (upper right) accumulator for furnishing source of current, with which the current from the hydrogen and calomel electrode is compared. The accumulator current is standardized by means of the standard cell.

(2) *By Means of the Potentiometer* (Fig. 24).—A standard potentiometer method in common use in biochemical laboratories of the United States will be described; it involves apparatus made in this

[1] Potassium chlorid is used, so that there will be as little potential difference set up at this junction as possible. K^+ moves at the rate of 64.6 units and Cl^- at 65.4; hence the low potential difference set up.

[2] Standardized by the voltmeter V, the null-point apparatus being the capillary electrometer or galvanometer, G. The voltmeter permits immediate determination of the reading, and hence a standard cell is not needed.

country and available at all times. Assuming that a determination is to be made of the hydrogen-ion concentration of a body fluid like blood, the procedure is as follows:[1]

(A) To standardize the storage battery against the standard cell W: Read the tag attached to the standard cell and set the figure, indicated on the tag, upon the dial in the upper left-hand corner of the potentiometer; this makes all future readings in terms of exactly one volt. Set the double knife-switch near the dial on "Std. Cell," which completes the circuit between the resistances of the potentiometer, the accumulator (storage battery), and the standard cell. Touch key "Res. 1" and note the deflection of the galvanom-

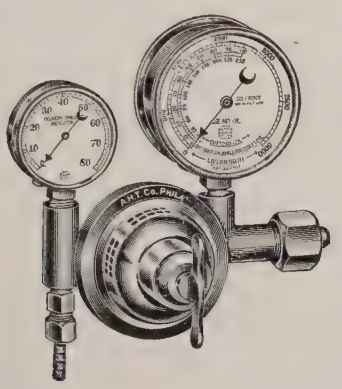


Fig. 25.—Details of gas-regulator for cylinders of H_2, etc. The flow of gas is regulated by means of the handle operating a diaphragm between the gauges. The larger gauge registers cylinder pressure; the smaller, pressure in the system passing to the bomb, or other apparatus.

eter streak of light. Now turn the nearer of the two milled heads at the right-hand end of the potentiometer box until, on touching the key just mentioned, there is but a small deflection of the galvanometer; the exact balance is finally made by adjusting the second milled head until there is no deflection of the galvanometer. Now try touching key "Res. 2;" there will be a slight deflection of the galvanometer, which is again adjusted, and, finally key "0" is touched and

[1] The instructions are for the Leeds and Northrup Type K potentiometer and the Clark scheme for accessories. It is supposed that the instrument and accessories have been carefully set up and tested against standard solutions of buffers (page 66). The whole apparatus should be enclosed in a thermostat for constant temperature. The thermostat may be a large box, insulated, with sleeve-holes for making the various adjustments.

the resistance is adjusted.[1] You now have the accumulator delivering exactly one volt current.

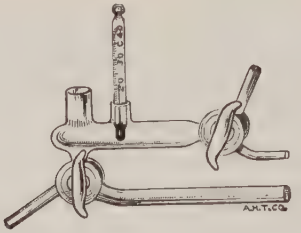


Fig. 26.—The Clark hydrogen electrode vessel. The electrode is placed in the opening to the left, above. The lower tubes below the cock are for drainage (right) and for connection with the liquid junction (left). The unknown is admitted from the upper right tube and hydrogen gas from the tube extending toward the right from the cock.

(B) To make the determination: Start the motor operating the hydrogen electrode vessels[2] (Figs. 26 and 27), and let the vessels tilt

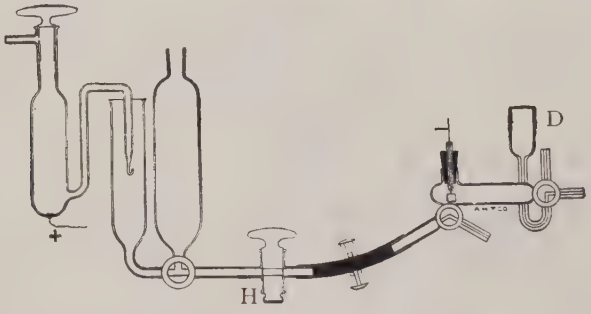


Fig. 27.—Detail of the apparatus for determining *p*H electrometrically. From left to right: Calomel half-cell; intermediate vessel containing KCl; KCl reservoir; liquid juncture; hydrogen electrode and its vessel. The electrode is shown as a small square inserted in the vessel.

away from the end containing the electrode.[3] Place some of the solution to be tested in the burette D at the opposite end of the vessel

[1] These three keys have smaller and smaller resistance to protect the instrument from too great potential differences to start with.

[2] It is customary to use two of these vessels for rapidity and accuracy.

[3] The electrodes should be blackened before the determination. They are first cleaned with chromic acid and then electroplated with platinum by means of a dry-cell and platinum dipping-electrode, the second electrode being the hydrogen electrode. Dissolve 2 gs. chlorplatinic acid ($H_2PtCl_6.6H_2O$) crystals in 10 mls. distilled water; use this solution for the blackening process. The deposit of platinum must be uniform and the effect a mossy black.

from the electrode and immediately before admitting the unknown fluid into the vessel, flood the vessel with hydrogen gas from the hydrogen cylinder, or other source of supply;[1] then fill the vessel with the unknown and again pass hydrogen gas into the vessel until it has displaced about half of the contents of the vessel. The electrode should be adjusted so that it is alternately bathed and exposed to the hydrogen atmosphere as the vessel is rocked by the motor. Adjust the column of connecting fluid KCl, first by letting some run through H and G to J, and then, by turning G appropriately, let the fluid come into contact with that in the electrode vessel.[2] Throw the knife switch to "E. M. F.," which completes the circuit from the accumulator through the potentiometer to the galvanometer and there brings it into contact with the current coming from the cell consisting of hydrogen and calomel electrodes. Start the motor and let it cause the hydrogen and H^+ in the solution to come into equilibrium. Then manipulate the dial in the middle of the potentiometer box until there is but a small deflection of the galvanometer when the key Res. 1 is depressed. Complete the balance by rotating the extended wire wound upon the drum. The readings on the dial are in tenths of a volt and those on the drum in thousandths of a volt (millivolt). Add together the two readings[3] and enter the result into your notebook. Note the temperature of the solution registered on the thermometer in the electrode vessel. Make three readings at five-minute intervals, and continue, if necessary, until uniform results are obtained.

Calculation of *p*H from E. M. F.—The E. M. F. having been determined in volts, by the method given above, the *p*H is obtained by the following formula:

$$p\mathrm{H} = \frac{\text{Observed volts} - 0.3377}{0.058523} \qquad \text{(XVI)}$$

[1] Hydrogen gas in cylinders under compression are obtainable. The gas is impure and must be freed from oxygen. The best procedure to effect this is to wash the gas through two gas washing bottles containing pyrogallol solution, and finally pass the hydrogen through a tube containing asbestos wool soaked with palladium hydrochlorid and then ignited; the palladium, as we have said previously, absorbs hydrogen and passes it, but oxygen does not pass. The tube should be heated electrically by winding around it a layer of nichrome wire connected with the house current through proper resistance.

[2] If the rubber tubing connecting the KCl vessel with the electrode vessel is pinched before opening the cock that admits the KCl to the electrode vessel, and then released after the cock is opened, a good intercommunication is established.

[3] Suppose the dial reads 0.6 and the drum 427. Then the total potential difference between the standard cell and the other cell (calomel and hydrogen electrodes) is 0.6427 volt or 642.7 millivolts.

This equation is obtained from a consideration of the procedure usually attributed to Nernst,[1] but in reality based on principles propounded by Gibbs (page 115, note 1). Returning to page 51, we may call the pressure exerted by the ions leaving the electrode as P (the Solution Pressure of Nernst) and their tendency to return to the electrode as p (the Osmotic Pressure of Nernst), analogous to the diffusion pressure of a crystal of permanganate (page 121) which becomes counteracted when the solution is saturated. The pressures are due to the kinetic action of the hydrogen ions and must obey the gas laws expressed as

$$PV = nRT \qquad \text{(XVII)}$$

in which R is the work performed per mol of gas at the temperature T (absolute) and n, the number of gram-mols of ions involved. If RT = PV, and RT is work, we may write

$$w = nRT \qquad \text{(XVIII)}$$

but in the hydrogen electrode, the work done is electromotive force (E. M. F.) and may be expressed in units of work (joules; 1 joule = 0.2389 gram (small) calories of heat); R becomes 8.316 joules per degree T. The work done results in the transfer of 1 mol of ions to the electrode involving 96,500 coulombs; that is,

$$E = \frac{8.316T}{96{,}500} \qquad \text{(XIX)}$$

But the activity of the ions at the two electrodes limits the work done and hence

$$E = \frac{8.316T}{96{,}500}\, n \frac{a}{a'} \qquad \text{(XX)}$$

in which a represents the activity of one electrode dipping into a normal solution of H^+ (page 54) and a' the activity of the electrode dipping into the unknown H^+ solution. However, a is unity, owing to the fact that it represents a normal solution of H^+. We have, then,

$$E = \frac{8.316T}{96{,}500} \ln \frac{1}{a'} \qquad \text{(XXI)}$$

or, since $\ln \frac{1}{a'} = -\ln a' = pH$

$$E = \frac{8.316T}{96{,}500}\, pH \qquad \text{(XXII)}$$

[1] Nernst, Walther, Director of the Physical-Chemical Institute, University of Göttingen, Germany

It is desirable to convert the logarithm from the Naperian (base$_e$) to common (base$_{10}$), logarithms which is accomplished by multiplying by 2.3026; then

$$E = 0.0001984T\ pH \qquad \text{(XXIII)}$$

or

$$pH = \frac{E}{0.0001984T} \qquad \text{(XXIV)}$$

If we use a calomel electrode in place of the normal hydrogen electrode (page 54), we must correct for the small electrode potential possessed by this element when used with a hydrogen electrode; that E. P. is 0.3377 when a decinormal KCl solution is used in making the element (note 5, page 54). Then we have:

$$pH = \frac{E_{obs} - E_{cal}}{0.0001984T} \qquad \text{(XXV)}$$

At 22° C., T (−273° C.) becomes 273° + 22° = 295.

Solving we have $pH = \frac{E_{obs} - 0.3377}{0.058523}$ (XXVI)

Compare equation page 59.

It will be observed that Sørensens' pH expression is not wholly arbitrary, but has a substantial basis (*cf.* page 39); it is the limiting expression of equation, XXI that is, $\ln \frac{1}{a}$ and represents the activity of hydrions:

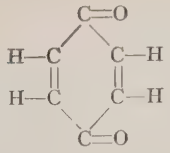
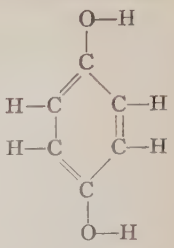
$$\ln \frac{1}{a} = -\ln a' = \qquad \text{(XXVII)}$$
$$-\log_{10} (2.3026)\ a'$$

since ln 10 = 2.30258 . . . . . etc.

pH Determination Using the Oxidation Reduction Potential Method.[1]—The Quinone Hydroquinon Electrode Principle.—On page 51, the origin of a potential difference when a metal is placed in a solution of its salts was described. Such a difference in potential arises if a metal (Pt, Au, etc.) be bathed in a mixture of ions, one set representing an oxidized substance and the other a reduced, such as, for example a solution of ferrous and ferric iron. It is to be understood that oxidation means "loss of electrons" and not necessarily a transfer of oxygen.

Now in place of using an inorganic substance like ferrous sulphate which has limitations such as entering the colloidal state, etc., when

[1] For a critical study of the oxidation-reduction method see Cullen, G. E. and Bülmann, E. Jour. Biol. Chem., vol. 64, page 727, 1925.

used electrometrically, it is possible to employ the organic substances quinon  (oxidized) and hydroquinon  (reduced) in exactly the same way. It is evident that *the difference between the two concerns a molecule of hydrogen.* If we place two beakers side by side and pour into one a given amount of a solution of quinone and into the second beaker, the same amount of solution of hydroquinon of identical ionic concentration, a unit fall of potential is established between the two electrodes:

$$C_6H_4O_2.H_2 \leftrightarrows C_6H_4O_2 + 2H + 2e$$

Hydroquinon. Quinon reduction 2 Coulombs.

As two hydrogen-ions involving two electrons are concerned in the above equation, it can readily be seen that the concentration of hydrogen ions is concerned in the production of the potential difference. The relation of the concentration of hydroquinon and of quinon is concerned in a similar way, so that the potential difference between the two electrodes is proportionate to the product of these two factors. In a fashion similar to that in which equation XX page 60 was derived we can show that this potential difference, E_H, is equal to a constant, $E_{\left(\frac{QH_2}{H_2}\right)}$, minus the product $\frac{RT}{nF}$ times the ln $\left[\frac{(QH_2)}{(H_2)} \times \frac{(H)^1}{(H)^2}\right]$, or, in equation:

$$E_H = E_{\left(\frac{QH_2}{H_2}\right)} - \frac{RT}{nF}\left[\ln\left(\frac{(QH_2)}{(H_2)} \cdot \frac{(H)^1}{(H)_2}\right)\right]$$

Expanding, we obtain

$$E_H = E_{\left(\frac{QH_2}{H_2}\right)} - \frac{RT}{nF} \ln \frac{(QH_2)}{(H_2)} + \frac{RT}{nF} \ln \frac{(H)^1}{(H)_2};$$

then, as $\frac{(QH_2)}{(H_2)} = 1$, since the concentrations were made equal in the quinon-hydroquinon solutions, the ln of the middle term is zero and this term drops out. Then replacing the term $\frac{RT}{nF}$ with its numerical

equivalent, remembering from the first equation that n = 2, and expanding as explained in equation XX, page 60, we obtain:

$$E_H = E_{\left(\frac{QH_2}{H_2}\right)} - 0.0001984T\,pH.$$

This is the working formula.

Reagents.—In place of using hydroquinon and quinon, the substance *quinhydron*[1] is used. This, in aqueous solution, dissociates, as shown in the formula, into quinon and hydroquinon in equimolecular equivalents.

Standard Sodium Acetate Solution.—By means of your 25 ml. pipette, place 100 mls. of standard normal acetic acid from the storeroom into a liter flask. Add 3 drops phenolphthalein indicator and add, from a burette, a solution of normal NaOH until a distinct, but delicate pink color appears. Then add 100 mls. of the normal acetic acid solution. Make to the mark with distilled water. The reaction of this solution should be *p*H 4.6. If the laboratory has a hydrion determination apparatus (page 56), this may be used for verifying the reaction.

The Electrode.—A plain platinum or gold (preferably the latter) electrode is used; it does not require platinizing. The electrode may be of foil, wire or gauze.

Procedure.[2]—Determination of the potential of the calomel electrode against the standard acetate solution: Pipette 3 mls. of the standard acetate solution into the electrode vessel (page 58). Add a few crystals of quinhydron, insert the electrode and mix, by shaking the vessel gently. Now make contact with the KCl liquid junction.[3] Adjust the potentiometer. Record the result as E. M. F. calomel-acetate. The calculation is given below.

The determination of the unknown: Repeat, using the unknown solution.

Calculation: Note the temperature and enter the table, page 64, to find the coulombs corresponding to this degree Centigrade. The

[1] This belongs to a group of quinones which Willstätter has called "meriquinoids." They have not been assigned structural formulae. For the present purpose we may give the following equilibrium formulae:—

$$\underset{\text{Quinhydrone}}{C_{12}H_{10}O_4} = \underset{\text{Quinon}}{C_6H_4O_2} + \underset{\text{Hydroquinon}}{C_6H_6O_2}$$

Quinhydrone is obtainable from the Eastman Kodak Co., as No. 217. The preparation from hydroquinon is given in the Appendix.

[2] The electrode must be clean. See page 58.

[3] It is not necessary to bubble hydrogen gas as in the platinized electrode procedure.

acidity of the acetate solution being known (pH 4.6), determine the E. M. F. of the acetate solution from the following equation:

$$E = E.\ M.\ F. + Y.\ pH$$

in which E. M. F. is the observed millivolt readings and Y the numerical value of the expression

$$\frac{R}{F \times 0.4343} \times 1000 \times T,$$

or

$$0.00019837T \cdot 1000$$

and calculated as follows for common temperatures:

15° C.	57.2 Value for Y
16.	57.4
17.	57.6
18.	57.8
19.	58.0
20.	58.2
21.	58.4
22.	58.6
23.	58.8
24.	59.0
25.	59.2
26.	59.4
27.	59.6
28.	59.8
29.	60.0
30.	60.2
31.	60.4
32.	60.6
33.	60.8
34.	61.0
35.	61.2
36.	61.4
37.	61.6

Finally, the unknown may be determined by the formula as given, that is, $E = E.\ M.\ F + Y.\ pH$ and solving for pH. Transposing:

$$pH = \frac{E.\ M.\ F. - E}{Y}.$$

Uses of the Oxidation-reduction Potential Determinations.—More and more the importance of the precise rôle oxidations and reductions are playing in biochemistry is being realized. As examples, we may give the following:

(1) The use of the method is a convenient one for pH determination.

(2) The evaluation of dyes to serve as indices of oxidation and reduction.

(3) The measurement of activity of oxidizing enzymes.

(4) The evaluation of oxidation in the heat destruction of vitamins.

Simplified Method for Determining *p*H. The Colorimetric Method of Sørensen.—By mixing solutions of electrolytes like acid and basic phosphates, NaH_2PO_4 and Na_2HPO_4, it is possible to secure sets of solutions which range from acidity to neutrality and from neutrality to alkalinity. If these solutions are made up carefully so as to determine the proportions of the two salts, it is possible to obtain a set of solutions which, when colored with certain dyes, or indicators, can be used as standards to determine the acidity or alkalinity of an unknown solution. An appropriate indicator will exhibit a range of colors each shade corresponding to a certain degree of acidity or alkalinity. Not all indicators, however, will produce this specific variation in colors, and the indicator must be selected with care. The salts used in these preparations permit a relatively large addition of acid or alkali before exhibiting a change in reaction, a phenomenon which we shall study in more detail later. If one were interested in the hydrion concentration around neutrality, the dye phenolsulphonephthalein[1] would be an excellent indicator, as it produces a finely graduated range of colors in solutions on either side of the neutral point. For ranges beyond the effective colors of phenol red other dyes are used.[2] Such solutions must be verified by the potentiometer method, but with care in obtaining pure ingredients and in weighing very accurate standards may be obtained. There are factors entering into the use of the colorimetric method for *p*H determinations which limit its usefulness; these are the presence of substances that alter the chemical composition of the dye; materials which interfere with the passage of light through the solutions, etc. In some cases it is possible to compensate for these difficulties, but in others recourse to the electrometric method must be made.

Limits of the Above Ranges.—For studies on the reaction of blood the phosphate mixtures and the three indicators—Methyl Red (*p*H (4.4–6.0)), Phenol Red (*p*H (6.8–8.4)), and Cresol Red (*p*H (7.2–8.8)) —are adequate (average normal *p*H of whole blood, 7.3), but if other body fluids, like gastric juice (*p*H 1.7), are under consideration,

[1] Called also phthalein and phenol red. The dye is used elsewhere in medicine, as in the detection of kidney lesions.

[2] For a complete list of dyes suitable for these purposes see Clark, W. M., The Determination of Hydrogen Ions, 2d ed., Baltimore, Williams & Wilkins, 1924. Associated with Lubs, these two investigators in the Government laboratories worked out a complete list of salt solutions and dyes for the colorimetric determination of hydrions. See page 66 for a list of dyes and solutions (Fig. 28).

these indicators and solutions must be supplemented by those giving greater range. For the gastric juice a more acid range must be used, with appropriate indicators. For the preparation of solutions and selection of indicators see Appendix.

EXERCISE 3.—Preparation of a set of standard solutions for blood *p*H. Secure from the store-room one-fifteenth molecular solutions of the basic salt, Na_2HPO_4[1] and of the acid salt, KH_2PO_4.[1] Arrange a series of 12 clean Pyrex[2] test-tubes sizes 125 × 15 mms. in

Fig. 28.—Clark-Lubs indicators. The dry substance is furnished in decigram amounts as shown above.

your test-tube rack. Provide each tube with a cork stopper that has been boiled in paraffin. Number each tube with a glass-marking pencil.[3] Now, using the Mohr 1 ml. pipette, graduated to tenths of ml. (see Fig. 229), and other pipettes as required, transfer accurately to Tube No. 1, exactly 0.25 ml. of Na_2HPO_4 solution;[4] to Tube 2, exactly 0.5 ml.; and so on until the 12 tubes have received their proper amounts. Clean the pipettes[5] first with tap-water from the

[1] Appendix.

[2] Pyrex glass is widely used in making baking-dishes and other ware which must resist high temperatures or the solvent action of reagents. It is manufactured by the Corning Glass Company, Corning, N. Y. The proper tube is Pyrex No. 46, lipless 125 × 15 mms.

[3] The best pencil for such use is a Century Negative Pencil for sale by the Eastman Kodak Co. It has a somewhat softer "lead" than the ordinary glass-marking pencil which is designed principally for marking commercial glassware and not the more delicate ware of the chemical laboratory.

[4] Some Mohr pipettes are graduated to the tip, while others are not. If the pipette belongs to the second class, it is necessary to exercise care in delivering small amounts and to remember that the zero is not at the end of the tip.

[5] Pipettes of the ordinary volumetric kind are so graduated that they either contain the amount indicated on them or deliver that amount when they are permitted to drain against the side of the vessel for about ten seconds. The first kind is called a "contain" pipette, the second, "deliver" or "tip-off" pipette. One should never blow through a pipette. If it is necessary to remove all the liquid (as in the contain pipette), hold one finger over the mouth-piece of the pipette and the warm hand around the bulb; the heat of the hand will cause the confined air to expand, driving out the last drop of liquid (see Appendix).

faucet, then with distilled water, and finally with a little of the next solution to be used,[1] discarding this washing solution. Now begin to place the KH_2PO_4 solution in the tubes, beginning with Tube 1, 9.75 mls. (5 mls. with the 5 ml. pipette, then 4 with the Ostwald-Folin

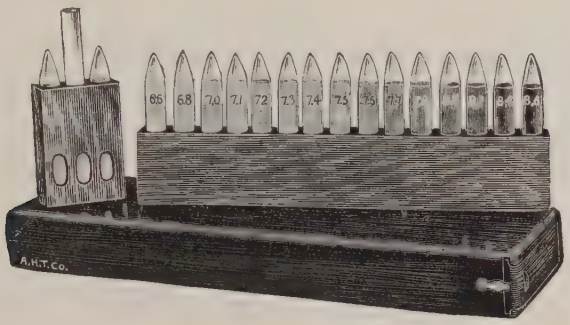


Fig. 29.—Set of solutions of known *p*H ranging from *p*H 6.6 (acid) to 8.6 (alkali), covering the range of reaction of blood and body fluids other than gastric juice and urine. To the left, a small comparison colorimeter. The unknown, in the middle test-tube, is compared with the nearest two tubes, placed one on either side.

1 ml. pipette and 0.75 with the Mohr 1 ml. pipette). Continue until all the 12 tubes are filled. Now to each tube add exactly 2 drops of the indicator as follows:

Tubes 1 to 4 inclusively	bromthymol blue
5 to 8	phenol red
9 to 12	cresol red

Mix by rolling the tube between the palms of the hands, then stopper each tube carefully, and invert it into an evaporating dish of paraffin on a water-bath so that the stopper and about half a centimeter of the tube are coated with the melted paraffin;[2] remove the tube and place upright in your rack to cool. Write the *p*H of each tube on a gummed label attached to the tube and make it permanent by paraffining. Compare your standards with those of the Department, and also note carefully whether the gradation of your colors is regular. Keep the tubes in your locker when not in use, for light causes a deterioration and change in color. The standards thus made are accurate for a year or sometimes longer.

Method of Withdrawing Blood (Fig. 32).—For the purpose of such determinations as the reaction of blood it is necessary to have

[1] Do not rinse the pipettes during the delivery of the Na_2HPO_4 solutions, because each solution is nearer in composition to the others than to water.

[2] Or one may use a solution of "hard" paraffin (melts at 60° C.) dissolved in carbon tetrachlorid, which is not inflammable.

at least 2 mls. of blood, which give about 1 ml. of plasma.[1] Blood is obtained in the adult by puncturing a vein ("venipuncture"), ordinarily the radial vein,[2] as it passes superficially[3] along the palmar sur-

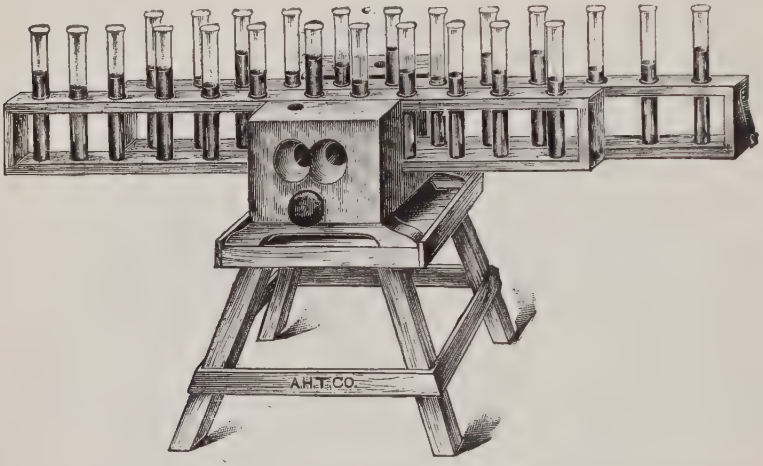


Fig. 30.—Comparison colorimeter for use with the standard buffer solutions for determining *p*H.

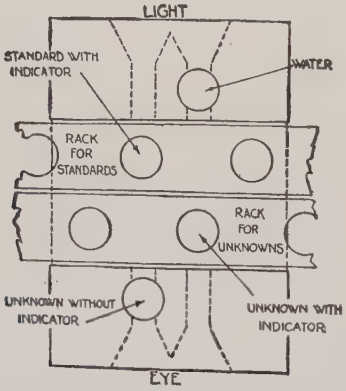


Fig. 31.—Details of the Cooledge colorimeter (Fig. 30). The view is from above. The tube without indicator is to compensate for differences of turbidity, etc.

[1] Corpuscle-free blood, unclotted; the clotted plasma is serum.

[2] Stadie suggests the use of arterial blood, derived from the radial artery and Frazer, Graham and Hilton, from the femoral. See Stadie, W. C., Journ. Exp. Med., vol. 30, page 215, 1919. Also Frazer, *et al.*: Jour. Physiology (English) vol. 59, page 221, 1924. Especial care is imperative in such manipulations and for class-work and for clinical diagnosis, venous blood is adequate.

[3] In obese subjects the vein is obscured. The best method of procedure in such cases is to adjust the collar of a plethysmograph around the biceps muscle portion of the arm and to pump up the sleeve to a degree that varies with the subject. By this means the

face of the forearm below the elbow (Fig. 32; also Fig. 238). Proceed as follows: Expose the subject's forearm and support it on a table. Dip a small pellet of absorbent cotton[1] into a 10 per cent. solution of iodin in 80 per cent. ethanol and wash the surface of the arm where the puncture is to be made. Now, using a sterile syringe[2] with No. 18 or

Fig. 32.—Showing method of obtaining blood from a vein. (From Todd, Clinical Diagnosis by Laboratory Methods.)

20 needle, push the plunger into the syringe as far as possible and then hold it ready to insert it into the blood-vessel. In an ordinary subject, press with your thumb against the vein so as to stop the flow of blood through the vein toward the heart; this will cause an engorgement of the blood in the vein and make it stand our prominently. Lay the

vein becomes palpable, if not visible. A final effort in locating the vein in difficult subjects is made by causing the arm to hang loosely from the shoulder and to have the subject open and close the fist. For arteriosclerotic cases, or in the older subject in which the skin is loose and permits the vein to move freely, place the first and second fingers, somewhat spread, across the vein and thus hold it while the needle is being inserted. The needle must be sharp. "There is, perhaps, no greater enemy to the blood chemist than a clinician armed with a blunt needle" (Beaumont and Dodds, Recent Advances in Medicine, page 2). A small carborundum stone should be kept ready for use. The bevel should be less acute than in the commercial needle (Fig. 246). Besides the radial vein, the median, median cephalic or other prominent vein may be used.

[1] It is not necessary that this cotton be sterile, for it is to be used with alcoholic tincture of iodin, which will sterilize it.

[2] For human subjects the all-glass Luer hypodermic syringe is the best, but for animal work the "Record" type (Fig. 33) is preferable. See Figs. 240 and 245.

needle obliquely to the surface of the arm, in the plane of the vein and directed toward the subject's body. Push the needle through the skin and through the upper wall of the vein, maintaining with the thumb of the hand holding the syringe a slight pull upon the plunger

Fig. 33.—A satisfactory syringe (Record). This syringe has a glass barrel and metal plunger. It is easily sterilized, durable, and works smoothly and accurately. (From Kolmer, Infection, Immunity, and Biologic Therapy.)

so that when the lumen of the vessel is reached the plunger will be easily moved upward, accompanied by blood. Do not withdraw the plunger with any but the slightest effort, for the blood must be

Fig. 34.—Donald D. Van Slyke, Member of the Rockefeller Institute, New York, N. Y. Responsible for numerous standard methods in analyses of blood-gases, dynamics of maintenance of neutrality, methods for analysis of protids, quantitative determination of amino-acids, etc. Extensive researches on the biochemistry of normal and pathological processes.

collected as it flows unaided. When the desired amount of blood has been obtained, remove the needle by withdrawing it in the same plane as it entered, wash with iodin solution, lay a piece of sterile gauze across the cut, and request the subject to hold it in place with

his free hand until bleeding has ceased. Immediately inject the blood from the syringe into a "half-test-tube" made by filing and breaking an ordinary 150 mm. test-tube in half; for class use the tube has previously been provided with about 10 mgs. of powdered crystalline potassium oxalate for every 5 mls. of blood.[1] Mix the oxalate with the blood thoroughly.[2]

EXERCISE 4.—Determination of the reaction of blood by the colorimetric method:

Place about 1 ml. of blood, drawn under oil, in a collodion tube[3] of about 2 mls. capacity. Tie the end of the tube with a piece of thread, leaving one end of the thread long enough to suspend the bag in 5 mls. of special saline,[4] in a test-tube that has been washed carefully with the saline. Stopper. Leave ten minutes and then remove the bag. Add to the dialyzate in the test-tube 5 drops of phenol red indicator[4] and mix. Compare the color of the solution with that of a set of standard phosphate mixtures[5] bearing phenol red as indicator. Repeat, using blood that has been oxalated as directed above, and compare the results with those obtained on unoxalated blood. Lastly, repeat, using blood that has had preservative (sodium fluorid and thymol) added, and compare results.

How the Blood Maintains Its Neutrality.—The blood of a healthy person shows a remarkable uniformity in reaction; indeed, there is but little variation even in disease. A reaction of *p*H 8 has been detected in certain cases of carcinoma, and immediately before death in severe diabetes a reaction of *p*H 6.9 has been found. The average normal reaction of venous blood is *p*H 7.32.[6] However, if one remove

[1] As recommended by Folin. See Laboratory Manual of Biological Chemistry, edition of 1925 with Supplement, New York, D. Appleton & Co., page 227.

[2] In collecting blood for the purpose of studying its reaction it is necessary to avoid contact with the air. Before the blood has been introduced into the half-test-tube pour about 2 mls. of paraffin oil into the tube, and when the needle is introduced, insert the tip below the surface of the oil. If the blood is to be kept for any length of time, a small amount of sodium fluorid mixed with thymol should be used; Sander (Wisconsin Psychiatric Institute, Mendota, near Madison) recommends a mixture of 50 milligrams NaF and 5 mgs. thymol for each 5 mls. of blood. See Sander, F. V., Jour. Biol. Chem., vol. 58, p. 1, 1923. Determinations made upon preserved blood are never satisfactory.

[3] For directions for making collodion tubes see Appendix. No anticoagulant is used in blood drawn for the purposes of studying its reaction, neutral K-oxalate giving *p*H 0.1 to 0.2 too high.

[4] Appendix.

[5] Page 66.

[6] See Van Slyke at end of chapter. Also Koehler, A. E., Brunquist, E. H., and Loevenhart, A. S., Jour. Biol. Chem., vol. 64, p. 313, 1925.

and measure the CO_2 of normal blood[1] it will be found that about 55 mls. of the gas may be collected from 100 mls. of blood.[2] If 55 mls. of CO_2 gas be bubbled through pure water, the reaction will be not *p*H 7.35, but *p*H 4.5. The following exercise demonstrates the power of sodium bicarbonate, which plays a major rôle in aiding the body to maintain its neutrality in the presence of acid, to compensate for the addition of acid:

Fig. 35.—Glenn E. Cullen, Professor of Biochemistry, Vanderbilt University, Nashville, Tenn. Identified with biochemical methods and analyses of physical chemical factors.

Exercise 5.—Place 10 mls. of a decinormal bicarbonate solution in a small beaker and 10 mls. of distilled water in a second, similar beaker. To each add 5 drops of indicator phenol red. Now titrate to *p*H 7.32 in both cases with hundredth normal HCl solution, comparing the colors with the standards. Note how many mls. are necessary to bring a decinormal $NaHCO_3$ to *p*H 7.32.[3]

The Explanation of the Regulation of Neutrality.—The key is given in the Appendix, where the Sørensen standards are described. A relatively small change in *p*H is obtained by adding salts in the

[1] The method is given on page 76.

[2] Reduced to standard temperature and pressure as described on page 79.

[3] Reference to the method of preparation of the indicator, phenol red, in the Appendix shows that when 5 drops of the indicator are added to distilled water they introduce a small amount of alkali, thus lowering the Ch of the solution. Then, when the HCl solution is added, it is possible to bring the distilled water solution up to *p*H 7.32.

middle of the set, but at either end a much greater difference is obtained by the addition of smaller amounts of salt. A quarter of a ml. of solution in the first tubes (0.50 − 0.25 = 0.25) causes a difference of *p*H 0.30, while four times that amount or 1 ml. in Tubes 7 and 8 causes a difference of but *p*H 0.16 (6.97 − 6.81 = 0.16). The reason for this is implied in the following principle: Ionization is decreased when more ions of the same kind are added.[1] The greater the ionization, the stronger the phosphate solutions will become, and hence anything that will retard ionization will maintain the reaction near the neutral point. At either end of the series of phosphate mixtures there is a relatively lower addition of ions, and hence there will be greater ionization. Similarly[2] the addition of relatively greater amounts of ions toward the middle of the series causes repression of ionization and hence a larger amount of acid or alkali may be added to the tubes in the middle of the series than in the first or last tubes. Such salt solutions are known as buffers,[3] and it should be observed that such salts are always those of inactive acid or base. A complete definition of a buffer is a salt of an inactive acid or base tending to hold the concentration of ions (H^+ or OH^-) to a constant value.

The Buffers of the Blood.—Two inorganic acids are involved, along with protid:

Carbonic acid, H—O—C(=O)—O—H, ionizing as H^+ and HCO_3^-.

The carbonates are characteristic of the blood plasma.

Phosphoric acid H—O—P(=O)(—O—H)—O—H

The phosphates are characteristic of the blood-cells.

[1] This is a simple expression of the Law of LeChatelier, Cf. Mellor.

[2] For example, compare the addition of phosphion in Tubes 7 and 8 (1 ml.) and in Tubes 11 and 12 (0.5 ml.). This phenomenon is expressed technically by saying: The greatest buffer value is gained when the dissociation constant equals the hydrogen-ion concentration, that is, $\frac{BA}{HA} = 1.0$, BA being a salt, and HA, a corresponding acid, and $C_H = K$; the expression K is described on page 41.

[3] From the analogy of a structure, such as is used in train terminals to oppose the movement of cars past certain points. In modern political parlance a "buffer state" is one standing between two rival states. Bayliss, some years before his death, suggested the term "tampon;" such an object is a roll of absorbent gauze laid upon some portion of the body for the purpose of absorbing liquid, as in "weeping" wounds. The analogy is a better one than "buffer," since it is the conception originally used by Sørensen and faultily translated into English as "buffer."

In the case of carbonic acid the "acid" salt, $NaHCO_3$,[1] is formed by the replacement of one hydrogen atom, H—O—C(=O)—O—Na. Both hydrogens may be replaced, yielding sodium carbonate: Na—O—C(=O)—O—Na,

but this salt plays a minor rôle in maintaining the reaction of the body; it is present in blood only to the extent of 0.5 per cent. because of the reaction $Na_2CO_3 + H_2CO_3 = 2NaHCO_3$. In the case of phosphoric acid there are three possibilities for salts:

Sodium di-hydrogen phosphate, $H—O—P(=O)(O—Na)(O—H)$ Primary

Disodium hydrogen phosphate, $H—O—P(=O)(O—Na)_2$ Secondary

Tri-sodium phosphate, $Na—O—P(=O)(O—Na)_2$ Tertiary

However, in the blood, the last is never realized, owing to its reversion to the secondary phosphate. Since it is immaterial whether sodium or potassium be used in such discussions, it is customary to abbreviate the term base, B, to designate any monovalent base, rather than to use a specific element (cation); hence we may speak of:

$KHCO_3$ or $NaHCO_3$ as $BHCO_3$
K_2CO_3 or Na_2CO_3 as B_2CO_3

and

KH_2PO_4 or NaH_2PO_4 as BH_2PO_4
K_2HPO_4 or Na_2HPO_4 as B_2HPO_4

The acid radicles like HCO_3^-, $HPO_4^=$, etc., are designed as A, so that

$NaHCO_3$	becomes in general formula				BA
Na_2HPO_4	"	"	"	"	B_2A
H_2CO_3	"	"	"	"	HA

The chief protid buffer of the blood is the coloring-matter, *hemoglobin*, a weak acid which exists either as oxyhemoglobin (more acid), or reduced hemoglobin, *i.e.*, simply hemoglobin (less acid).

Oxyhemoglobin	becomes in general formula				$HHbO_2$ (free oxyhemoglobin)
The basic salt	"	"	"	"	BHbO

It is this basic salt that is responsible for three quarters of the power of the blood to transport carbon dioxid from the tissues to the lungs

[1] Sodium is used through this discussion for simplicity, but potassium and perhaps other cations may form similar salts. Sodium salts are by far the most important.

through the venous blood. The buffers of the blood are, then, $BHCO_3$, B_2HPO_4, and BHbO. These three substances comprise the alkali reserve of the blood which is utilized to neutralize acids that are given to the blood by the tissues. Acidosis is that condition in which the alkali reserve is abnormally low, and hence inadequate to carry as much acid as the body demands for health.[1]

The Reaction of Blood.—It is so easy to form misconceptions concerning the relation between acids and bases in the blood that we offer the following statements to help the reader to avoid pitfalls:

(1) The reaction in normal man is always slightly alkaline; considering *p*H 7 as neutrality, the reaction of arterial blood is *p*H 7.35, that of venous blood *p*H 7.32.

(2) The reaction of the blood in disease rarely encroaches on the acid side of neutrality, and when it does, death inevitably ensues. A reaction of 6.92 has been reported in patients nearing death.

(3) In acidosis the blood never becomes acid save under the conditions spoken of in (2) above; such conditions are called "uncompensated acidosis."[2] Hence *p*H determinations of blood show little variations and are of no value in acidosis as single estimations.

(4) The reason why, even in acidosis, the blood does not become acid is because of the protective action of the alkali reserve made up of the three factors previously mentioned—the carbonate, phosphate, and protid, which act like a sponge, chemically soaking up the acid.[3] When the sponge becomes so saturated or fixed with acid that it can accept no more, death ensues. For the manner in which the alkali reserve is depleted see page 81.

The Measurement of the Alkali Reserve.—There are several methods of measuring the alkali reserve. The one commonly used in clinical medicine at the present time is the Van Slyke method[4] which is based upon the following principles:

[1] For a complete treatment of the maintenance of neutrality in body fluids see Henderson, L. J. Journ. Biol. Chem., vol. 41, page 401, 1920. Also: Austin, J. H. and Cullen, G. E. Hydrogen Ion Concentration of the Blood. Baltimore, Williams and Wilkins, 1927.

[2] Compensation of acidosis is done by the respiration; increased ventilation of the ungs removes CO_2, thus saving the alkali reserve.

[3] See page 86.

[4] The apparatus used in this determination is also used for other analyses. In Fig. 130 is a description of an improved form of the vacuum extraction apparatus, which may be used for the determination of alkali reserve.

Demonstration.—*The Van Slyke Method.*—Principle: Carbon dioxid in the presence of water becomes the organic acid $C{<}\begin{matrix}O-H\\O-H\end{matrix}$ which ionizes as the monovalent H^+ and HCO_3^-. It may ionize farther as a bivalent acid, H^+, H^+ and $CO^{=}_3$, but the former is the important form in this discussion. The anion HCO_3^- is capable of reacting with the alkali reserve of the blood; thus we have B or B_2 as cations, and these form, with the anion HCO_3^-, such substance as $NaHCO_3$. Carbon dioxid is chemically bound in this manner in the blood. In addition to this carbonate method of binding CO_2, there is the binding of the acid by the hemoglobin.[1] Some CO_2 is also bound by the plasma protids.[2] If, then, we desire to determine the amount of alkali in the blood, we must determine how much CO_2 gas these various factors will accommodate. The blood is saturated with CO_2 derived from a cylinder of compressed CO_2 such as the proprietor of a soda fountain uses;[3] or the breath of a normal person may be used. To accomplish this the blood is spread upon a surface in a thin layer and exposed to the gas; then a measured quantity of the saturated blood[4] is subjected to a Torricellian vacuum[5] produced in the Van Slyke apparatus (Fig. 36).

Procedure.—Into the burette, A, introduce 2 mls. dilute ammonium hydroxid solution. With the leveling bulb J held below B, open B to permit the hydroxid, but no air, to enter C. Then raise J above the top of A and turn B until communication is made between C and D, expelling the hydroxid solution. Permit a small amount of mercury to remain in D to act as seal. Place about 1 ml. of albolene in A. The instrument is now ready for use. Saturate 2 mls. or more of blood plasma[6] with CO_2 by pipetting it, without exposure to the air, into a separatory funnel, the tube of which communicates with a column of glass beads in a bottle; the bottle is provided with a mouth-

[1] Page 83.

[2] Page 83.

[3] If such a source is employed, the tension of the CO_2 gas must be adjusted to about the average of that in the human breath, that is, 40 mms. of mercury.

[4] Or plasma; plasma is used frequently, because the amount of CO_2 bound by the corpuscles is proportionate to the amount bound by the alkali of the plasma, and the manipulation of the plasma is much easier than whole blood.

[5] A vacuum is established similar to that overlying the mercury of a barometer.

[6] The plasma has been obtained by the method given on page 69, followed by centrifuging. The plasma layer is about one-half of the total volume of blood. The plasma is carefully pipetted from beneath the oil into the separatory funnel mentioned in the text.

piece. After rotating the funnel to apply the blood in a thin layer over its inner surface, so that it may come into immediate contact with the CO_2, breathe, naturally, through the column of beads and thence through the funnel, the breath passing out the mouth of the funnel. The excess moisture of the breath is thus condensed upon the beads and does not dilute the blood. Pipette by means of an Ostwald-Folin 1- or 2-ml. pipette (Fig. 37) exactly 1.0 or 2.0 mls.

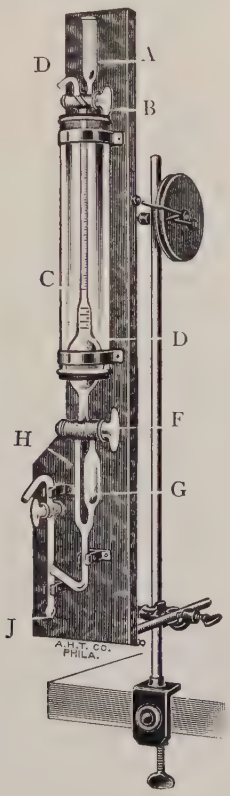


Fig. 36.—Van Slyke apparatus for determining the CO_2-combining power of blood in studies of acidosis. Rubber tubing of the leveling bulb is attached to J.

of the blood into the burette, placing the tip of the pipette beneath the surface of the oil to avoid loss of CO_2. Place J low and carefully permit the blood to flow into C by turning B appropriately. Add 3 drops caprylic alcohol to the burette and let this follow the blood. Permit no air to enter the apparatus. Finally add enough 5 per cent. H_2SO_4 solution to the burette and thence to the graduated tube to bring the reading to exactly 2.5 mls. Place a drop of mercury in the burette to act as a seal. Now lower J below the mercury level in a

barometer,[1] thus establishing a vacuum in which the CO_2 gas leaves the substances with which it has been chemically bound and exists as a free gas. Permit the mercury to fall to F and close the cock before any of the blood leaves D. Before the column of CO_2 gas is

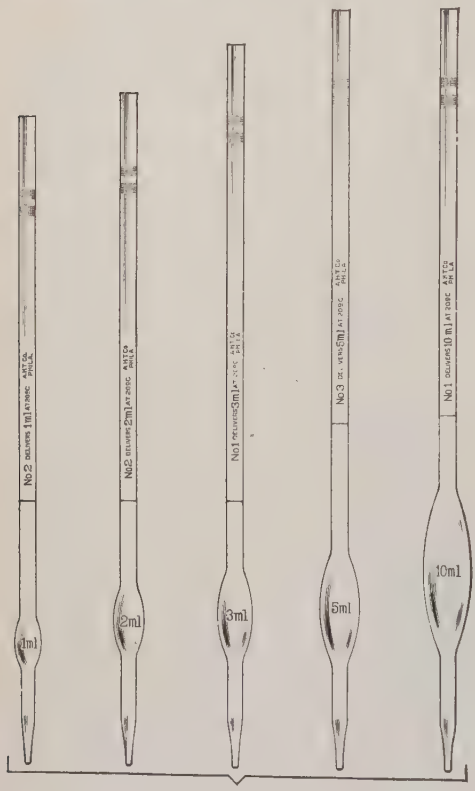


Fig. 37.—The Ostwald-Folin type of pipette widely used in biochemical work.

Fig. 38.—Van Slyke blood pipette.

measured the liquid portion of the blood must be disposed of; open F and permit the blood to pass into G. No gas may accompany the blood.[2] Raise J above B and then turn F until the mercury from H

[1] Average barometer reading at sea-level (Massachusetts General Hospital, Boston, for instance, 760 mms.; Philadelphia, Washington, and similarly located cities, about 755 mms.; cities lying in the great valleys, like Chicago, 750 mms. The weather bureau maps are reduced to sea-level readings. A simple form of barometer for gasometric work, such as this determination and the gasometric method for amino-nitrogen (Fig. 113), is the siphon barometer shown in Fig. 118. In the present instance, of course, it is not necessary to use a barometer, but the manipulation is made easier if the excursions of the leveling bulb be maintained within the limits of the barometric reading for the locality. The barometer is used, however, in the calculation.

[2] Except the negligible amount dissolved in the blood.

passes into D and thence into C, carrying with it the column of CO_2 gas. Bring the surface of the mercury in the level attached to the mercury reservoir J into the same horizontal plane as the meniscus of the mercury in C and read the graduation on the calibrated tube at this plane.

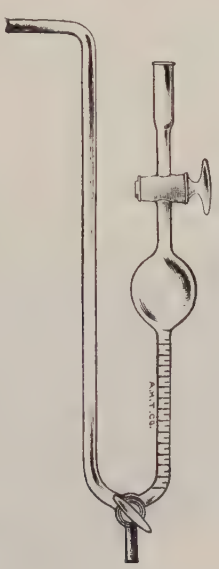


Fig. 39.—The Fridericia alveolar air method for acidosis. The patient respires through the mouth-piece (upper right). The breath is trapped by closing the stop-cock below the mouth-piece and also the lower cock. A rubber bulb is attached to the left-hand arm of the tube, the lower tip below the cock just mentioned dipped into 20 per cent. NaOH solution, the cock turned to admit the alkali into the left arm, and then by appropriate turning of the lower cock aided by the rubber bulb, about 3 mls. of the alkali are permitted to flow into the right arm. The cock is closed, the instrument shaken, immersed in water for constant temperature, the lower cock turned to admit water into the right-hand arm, and the percentage CO_2 read on the scale. The method is less accurate than others.

Calculation.—As in all gas analysis, it is necessary to express results in standard measurements. The volume of a gas varies inversely with the pressure (Boyle's law) and directly with the temperature (Charles' law). The barometer stands, on the average, at sea-level, 760 mm. of mercury and zero of the Absolute Scale is $-273°$ C. Then:

$$\text{Burette reading} \times \frac{\text{Barometric reading}}{760} \times \frac{273}{273 + \text{temperature}} \times 100 \quad \text{(XXVIII)}$$

gives the CO_2 bound by 100 mls. of blood.[1]

[1] If 1 ml. of blood was used.

Tables for rapid conversion of the data obtained by this method are given in Appendix. The average normal figure is 55 to 65 mls. CO_2 per 100 mls. of blood.

What Causes Variation in the Alkali Reserve?— Acidosis.—The body is continually producing acids of various kinds Each of the three typical kinds of food (starches, fats, and protids) give rise to CO_2 when they undergo change in the body Much of this acid is exhaled from the lungs as CO_2 gas, and some is excreted through the

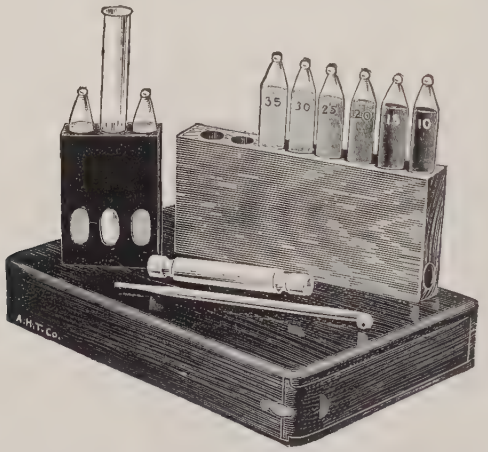


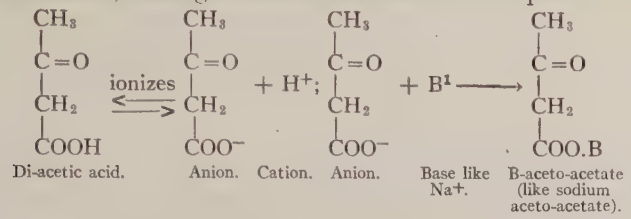
Fig. 40.—The Marriott method for detection of acidosis by alveolar air CO_2. The patient breathes back and forth into a football bladder previously filled with 600 mls. of air. The contents of the bladder are then bubbled through a buffer solution (in the tall test-tube) and the resulting color is matched with one from the set. The calibrations are in mms. Hg. alveolar air pressure. Normal is 35 to 40.

kidneys in conjugated form.[1] This conjugated CO_2 is fixed and does not reappear as gaseous CO_2 capable of being eliminated by the lungs. In much the same way, certain acids, when present in abnormal amounts, owing to derangement of the oxidizing powers of the body, hold the alkali reserve in conjugated "fixed" form. Such derangement occurs in the lessened oxygen tension on high mountains, and in the lowered efficiency of body oxidation in diabetes mellitus. Four-carbon acids, like the hydroxy-acid beta-hydroxy-butyric acid, $CH_3.CHOH.CH_2.COOH$; the keto-acid, aceto-acetic acid[2] CH_3.

[1] As, for example, in urea, $H{-}N = C\begin{matrix}O\\ \\ NH_3\end{matrix}$; uric acid $\begin{matrix}HN{-}C{=}O\\ \quad\;|\quad\;|\\ O{=}C\quad C{-}N{-}H\\ \quad\;|\quad\;\|\quad\;>C{=}O\\ HN{-}C{-}N{-}H\end{matrix}$

[2] Also known as di-acetic and acetyl-acetic acid.

$C=O.CH_2.COOH$ arise in diabetes mellitus and these react with the alkali reserve; taking di-acetic acid as an example:



The alkali reserve (B) is exhausted by being thus "fixed" and eliminated through the urine, and CO_2, being unable to be carried through the blood, accumulates in the tissues.[2] Consequently, the Van Slyke method for chemically bound CO_2 shows a lowered reserve in acidosis; that is, a lower combining power of the blood for CO_2.[3]

Characteristics of the Alkali Reserve.—The carbonate is characteristic of the blood plasma and the phosphates of the cell. At the normal reaction of the blood (*p*H 7.3) the phosphates are more efficient than the carbonates in taking care of the acids. The carbonates represent 6 per cent. of the total alkali reserve as measured by the ability of the blood to transport CO_2; the phosphates, 25 per cent. A glance at the table of Sørensen phosphate buffer mixtures[4] reveals the fact that when $\frac{BA}{HA} = 1.0$,[5] buffers are most efficient, that is, in the table, the least change in reaction (*p*H) occurs where $KH_2PO_4 = Na_2HPO_4$. In the blood, however, at ordinary *p*H, 7.3, the corresponding carbonate ratio $\frac{BHCO_3}{H_2CO_3} = \frac{20}{1}$, a ratio quite different from unity, but which approaches more nearly to unity when the *p*H of the blood becomes more on the acid side of the normal blood reaction *p*H 7.3. When the reaction of blood approaches a point which is clinically critical (more acid than *p*H 7.33; see page 75), the bicarbonate is at its theoretically maximum efficiency in neutralizing carbonic

[1] Representing base as explained on page 74.

[2] The result is an interference with oxidative processes in the tissues. See Koehler. A. E., Behnemann, H. M. F. and Loevenhart, A. S., Amer. J. Physiol., vol. 74, p. 590, 1925,

[3] A homely illustration of the depletion of alkali reserve in acidosis may be given: Persons, representing the CO_2, are waiting on the corner for a street-car; ordinarily there are plenty of seats (alkali reserve) in the cars, but on this occasion (acidosis) the seats have been occupied before the car arrives. The persons in the seats represent the fixed acids which crowd out the CO_2. In order to determine the total seating capacity (CO_2 combining power), one might fill the empty seats during ordinary times and count the number of persons filling the seats after requesting all of them to assemble outside the car; this represents the Van Slyke procedure which fills up the alkali reserve with CO_2 and then withdraws it for the purpose of estimating its amount.

[4] Appendix.

[5] Page 73.

acid. At pH 6.95 the phosphates are also at their greatest efficiency, but they always work at a ratio more nearly that of unity than the carbonates; for when the ratio $\frac{B_2HPO_4}{BH_2PO_4} = 1.0$, the pH is 6.8 as compared to the pH 6.1 when the bicarbonate ratio $\frac{BHCO_3}{H_2CO_3} = 1.0$. It is interesting to note that of all the various possible buffers which might have been adopted by the organism to take care of acid, the carbonate and phosphate work at greater efficiency at the ordinary normal blood reaction, namely, near neutrality.

What is the Magnitude of Gain in Buffer Efficiency?—We have stated that the alkali reserve becomes more efficient as the acidity of the blood increases, and the question may be asked, How much more acid than is normal could the buffers of the blood accommodate under extreme conditions? Using the figures of Van Slyke,[1] when the change is from the normal alkalinity of the blood (pH 7.35) to pH

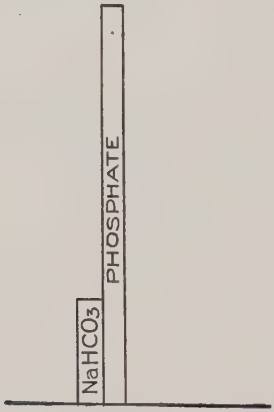


Fig. 41.—Relative buffer value of bicarbonate and phosphate; as the pH tends to rise in body fluids, the $NaHCO_3$ efficiency rises.

7.25, 1.67 mls. of H_2CO_3[2] could be added for every 100 mls. of blood to neutralize the increased alkalinity produced by the greater efficiency of the buffers. Phosphate is largely responsible for the buffer effect that permits an additional 1.67 mls. of H_2CO_3 to be added to blood; bicarbonate has a less important buffer action. It is seen that the buffering of the phosphate is highly efficient.

Behavior of the Phosphate in Buffer Action.—The fundamental equation is:

$$B_2HPO_4 + HA = BH_2PO_4 + BA \qquad \text{(XXIX)}$$

As an example we may use:

$$Na_2HPO_4 + H_2CO_3 = NaH_2PO_4 + NaHCO_3 \qquad \text{(XXX)}$$

[1] Van Slyke, D. D., Physiological Reviews, vol. 1, p. 141, 1921.

[2] Or, considering the CO_2 as bicarbonate, then 9.86 mls. of bicarbonate CO_2.

The phosphate BH_2PO_4 is passed into the urine and lost from the body; this necessitates continued replenishment from the food.

What Share Has the Cell in Buffering?—It is evident from the above discussion that the cell is rich in buffer substance. Under certain conditions the cells impart to the plasma of the blood some of their buffer value, becoming indirectly the chief reservoir of alkali. The alkali of the cell is composed of phosphate and protid. The protid in turn consists of hemoglobin and stroma protid. The hemoglobin is by far the more important:

	Per cent.
Total buffer power of the cell protid	94
Due to hemoglobin as a buffer-salt	9
Due to hemoglobin as changed from more acid hemoglobin to less (page 84)	85[1]

In this analysis the hemoglobin is given full credit for all the buffering of the cell so far as protid is concerned. We shall explain in detail, presently, how the hemoglobin acts as buffer. Considering the total cell buffer value:

	Per cent.
Total buffer value of the blood	100
Due to plasma buffers	16
Due to hemoglobin	62
Due to phosphate	22[2]

From this table the phosphate appears to be responsible for about one-quarter of the total buffer value of the blood.

How the Hemoglobin Furnishes Alkali (Fig. 42).—We have just seen that the hemoglobin has a dual capacity in furnishing alkali to the blood: (1) by means of its buffering power, and (2) by means of change in reaction. The red coloring-matter of the blood is a salt,[3] but it possesses an acidity, inasmuch as the acidic properties of this (the coloring-matter of the substance) preponderate over the basic nature (of the protid part of the substance). When the compound is oxidized it imparts to the blood a normal reaction of *p*H 7.33 (lungs); but when it is reduced (tissues), it is less acid (*p*H 7.35). This property of becoming more alkaline[4] (by *p*H 0.02) leads to its important rôle in buffer action. A reaction differ-

[1] Van Slyke, Physiol. Revs., vol. 1, p. 562, 1921. The figures are from the subject "J. J." studied by two English biochemists, Joffe and Poulton.

[2] These figures are compiled from Van Slyke's evaluation of the case "J. J." They differ from those given by Van Slyke in that they are calculated from 100 per cent. rather than the total addition percentage obtained by adding together the quantities obtained by Poulton, who analyzed the blood of "J. J."

[3] Page 382.

[4] That is, less acid; the substance is always acid.

ence of *p*H 0.02 may seem inadequate to account for such profound changes in the buffer value of the blood, but in actual experiment,[1] of the total 8 mls. CO_2 carried in every 100 mls. of blood, the reaction of which is beween *p*H 7.35 and 7.33, hemoglobin is responsible for 5.8 mls. per 100 mls. blood. Recent studies, moreover, have

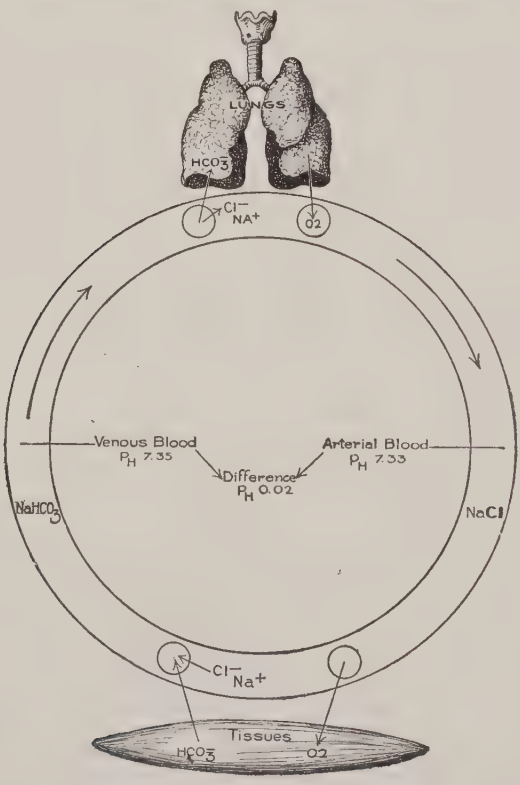


Fig. 42.—The "chlorid shift" in the blood. In the lung hemoglobin becomes oxygenated (O_2) and is more acid; in the tissues the hemoglobin becomes reduced and more alkaline. Anions tend to enter the erythrocyte (HCO_3^-; Cl^-); Na^+ remains in the plasma and receives HCO_3^- to form the bicarbonate, $NaHCO_3$. When oxidized in the lungs, the erythrocyte becomes more acid, causing anions (HCO_3^-; Cl^-) to leave the erythrocyte. The volatile CO_2 passes from the blood, while the Cl^- links with Na^+ to form NaCl.

shown that, since hemoglobin is amphoteric (page 108, note.) it may unite, chemically with—HCO_3^- to form a salt, $HbO.HCO_3$. It is probable, however, that this is inconsiderable as a function of the buffering effect of hemoglobin. We shall speak on page 383 of another function of hemoglobin.

[1] See Van Slyke's Physiol. Revs. article, p. 545. Also Campbell, J. M. H., and Poulton, E. P., Journal of Physiol. (English), vol. 54, p. 152, 1920.

Consequences of the Increase in Alkaline Properties of Reduced Hemoglobin.—In the tissues the blood loses oxygen, and consequently the oxyhemoglobin becomes reduced hemoglobin. During this process, as we have seen, the hemoglobin becomes less acid by *p*H 0.02. This basicity of the hemoglobin must be compensated, which might be done by the hemoglobin leaving the corpuscle and becoming neutralized in the blood or plasma; or by acid substances entering the corpuscle and neutralizing the alkalinity there. The latter is the case, for hemoglobin leaves the cell only in pathological states.

The Anion Shift (Chlorid Shift).—Hemoglobin cannot leave the cell and sodium cannot enter it;[1] but anions, like HCO_3^-, HSO_4^-, and especially Cl^-, may and do pass into the corpuscle to aid in neutralizing the alkalinity. Of these the chlorion is the most important; it is obtained from the dissociation of sodium chlorid:

$$NaCl \rightleftarrows Na^+ + Cl^-.$$

When the anion Cl^- is absorbed into the corpuscle, the cation Na^+ is free to unite with CO_2 from the tissues: $Na^+ + HCO_3^- \leftrightarrows NaHCO_3$. The possibilities of ionic reaction under these conditions, all instigated by the reduction of oxyhemoglobin, are of the nature of an endowment, or loan of alkali by the cell to the plasma and the following reactions give the combinations:

Plasma.	Cell wall.	Cell interior.
(1) $NaCl + H_2CO_3 \rightleftarrows NaHCO_3 + HCl$	$HCl \longrightarrow$	$HCl + K_2HPO_4 \rightleftarrows KH_2PO_4 + KCl$
		$HCl + KHbO_2 \rightleftarrows HHbO_2 + KCl$
(2) Na-protid salt + H_2CO_3 $\rightleftarrows NaHCO_3$ + H-protid		$HCl + KHbO \rightleftarrows HHbO + KCl$
	$H_2CO_3 \longrightarrow$	$K_2HPO_4 + H_2CO_3 \rightleftarrows KH_2PO_4 + KHCO_3$
		$KHbO_2 + H_2CO_3 \rightleftarrows HHbO_2 + KHCO_3$
		$KHbO + H_2CO_3 \rightleftarrows HHbO + KHCO_3$

Scheme of ion behavior in blood reactions.

Sulphion does not play a rôle of any importance; this ion does not readily pass through membranes.[2] In the capillaries of the lungs NaCl is regenerated since the oxidation of HbO by the oxygen within the lungs causes increased acidity in the corpuscle and the acid-producing ions HCO_3 , Cl , etc., leave the cell. The anion HCO_3 is

[1] Repeated analyses of blood-corpuscles fail to show the presence of sodium.

[2] This quality is employed in catharsis when magnesium sulphate (Epsom salts) is used. See page 124.

volatile and passes off, Cl^- remaining in the blood to form NaCl with the Na^+ freed from the bicarbonate, $NaHCO_3$. It is assumed that by the above equations involving NaCl and carbonic acid, some HCl is formed which, typically of all acids, increases the permeability of the corpuscle and permits its passage into the erythrocyte, in which it removes base attached to the hemoglobin and to the phosphion. The result is an increase in $—Cl^-$ in the corpuscles, that in the plasma being diminished; also, there is an increase of $NaHCO_3$ in the plasma (equation 1, page 85).

Summary for Buffers.—The blood, being a transport system, must maintain its neutrality and serve as a means of carrying CO_2 from the tissues to the lungs where it is exhaled through the breath. The chief agent producing the alkali for this purpose is the red coloring-matter of the blood-corpuscle, hemoglobin, abbreviated $HHbO_2$ for oxyhemoglobin of arterial blood and HHbO for reduced hemoglobin of venous blood. During the process of reduction of hemoglobin in the capillaries of the tissues it becomes less acid (more alkaline) and this alkalinity is the greater portion of the alkali reserve. The alkali reserve is capable of chemically binding the carbon dioxid which arises from the processes of burning in the tissues. The CO_2 is carried in the venous blood as bicarbonate, as a part of hemoglobin, and in other ways of lesser importance. In the lungs, the CO_2 passes from the corpuscle and enters the alveoli of the lung, whence it passes to the outside air. At the same time that CO_2 leaves the corpuscle, other substances that are acidic in nature, like Cl^-, also depart into the plasma. The Cl^- is reorganized into NaCl from the Na^+ that was freed from the bicarbonate when CO_2 passed into the alveoli. This latter process (and accompanying ion shifts) is known as the chlorid shift or, better, the anion shift.[1] In these ways the blood is able to transport carbon dioxid from the tissues. In acidosis the alkali reserve is "fixed" by non-volatile acids, and CO_2 remains in the tissues, causing, in severe cases, unconsciousness and, ultimately, death.

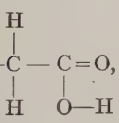
Accessory Factors Regulating Reaction of the Blood.—When one starts to exercise after a period of repose his breathing is accelerated. This is due to a temporary lack of compensation and an increase in the acidity of the blood,[2] owing to the sudden increase in the amount of CO_2 produced by the tissues. There is a center in the medulla in the brain, known as the respiratory center, which responds to small changes in the reaction of the blood. Whenever an increase

[1] Attempts to show that the cations like Na^+ play a part in such regulation of neutrality have not met with success.

[2] Decreased alkalinity.

in hydrion concentration occurs, this center is stimulated, impulses are sent to the ventilating apparatus of the lungs,[1] and increased ventilation ensues. On the other hand, too much ventilation, as with forced breathing, leads to apnea[2] because the CO_2 is pumped out of the lungs, leaving a decreased hydrion content in the blood, with consequent lack of stimulation of the respiratory center. The alkalosis observed in inexperienced mountain climbers is due to this "Auspumpung,"[3] which has been described by German and English investigators, and also by American physiologists.[4] The kidney compensates for this alkalosis by excreting more base.

Momentary Acidity and Titrable Acidity.—Throughout the preceding discussions we have purposely not mentioned titration, the common method for determining reaction of fluids in the general chemical laboratory. We shall now explain why this method has a limited application, especially in biochemistry: Titration determines the total amount of acidity which can be obtained from a given substance *if ionization were complete.* But ionization is not complete under ordinary circumstances. Moreover, ionization differs with different substances,[5] a decinormal solution of a strong acid, like HCl (*p*H 1.07) having a higher acidity than a weak one like acetic acid (*p*H 2.87), yet titration gives the same figure in each case, for the term "decinormal" means one-tenth the number of hydrogen atoms replaceable with a metal like Na, existing in a liter of normal solution. Not all of the hydrogen ions are replaceable; thus, in acetic acid,



the H's in the methyl group, CH_3 cannot be replaced by a metal, while the carboxyl-hydrogen is replaceable; but even this H is bound ten times as tightly to the oxygen as the H in HCl is bound to the Cl. It is for this reason that titration seldom gives the desired answer. If it is necessary to determine the total amount of acidity obtainable from a given solution of acid, titration will give this figure. If, however, it is essential to know how much H^+ is free from the carboxyl, hydrion concentration determination must be made. The acid of the stomach, HCl, is a good example. If the acid in a sample of gastric juice is titrated, in the ordinary way,

[1] Thoracic muscles and diaphragm.

[2] Gr. *a*, without, and *pnea*, breath; breathing is suspended.

[3] German for "pumping out."

[4] Schneider, E. C., special investigator, U. S. Aviation Field, New York. His studies have been made largely on Pike's Peak, Colorado, and at Mitchell Field, New York.

[5] Page 43.

a certain amount of hydrogen is found to be replaceable by the metal used (Na, for example, in NaOH), which represents 100 per cent. ionization as the successive stages of titration are accomplished.[1] However, in the stomach under normal conditions ionization occurs only about 81 per cent., and consequently titration would indicate about one-sixth more acidity than actually occurs physiologically. If we attempt to replace HCl with sulphuric acid in the same normality (about decinormal), we find that the gastric secretion does not digest food for two reasons: (1) the sulphion is not as effective with the enzyme pepsin which causes gastric digestion; and (2) (which is important in the present discussion), sulphuric acid is a weaker acid than hydrochloric, ionizing a third less than HCl. Therefore, about 62 per cent. more acid is found by titrating H_2SO_4 of the same normality than actually exists, or, to be more exact, than is capable of acting in digestion. However, for some comparative purposes, in gastric analysis,[2] titration serves in place of hydrion concentration determinations.

The *relative merits of hydrion concentration determinations and titration* for practical biochemical work may be presented as follows:

It is desirable to utilize:

Hydrion determinations.

In all cases in which reaction affects physiological action, as in the activity of enzymes, maintenance of blood and tissue reactions, etc.

In considerations of growth of micro-organisms, like bacteria, fungi, etc., and especially in studies concerning infection.

Pharmacological effects, depending upon the actual (momentary) acidity at the time of administering the agent. Thus the urinary antiseptic hexa-methylene-tetramin[3] is efficacious, as Hanzlik[4] has shown only when the *p*H 5, at which reaction, formaldehyde, H.CHO, is formed and acts as the antiseptic. Consequently this substance has no value except when used within the hydrion limits given above.

Titration.

In reactions of body fluids in which no especial correlation exists between (H^+) and physiological action, as in the case of the urine, in which the reaction is a function of the acid-base intake of the food and the excretion of phosphates.

In chemical determinations involving mass-volume reactions such as one encounters in determinations of substances in biochemical material: The determination of nitrogen by the Kjeldahl[5] method, etc. In cases in which comparative rather than actual results are required, as in clinical expressions of gastric acidity, which run parallel by the two methods, (H^+) and titration.

[1] However, by using suitable indicators, the titration method gives figures comparable to CH data. See page 445.

[2] Page 446, buffer value.

[3] Commercially known as "urotropin" after the German preparation of Schering and Glatz.

[4] P. J. Hanzlik, Professor of Pharmacology, School of Medicine, Leland Stanford, Jr., University, San Francisco, California. See Jour. Amer. Med. Assoc., vol. 54, p. 1140, 1910; also vol. 62, p. 295, 1914.

[5] Page 287.

By means of indicators, properly selected, titration results are convertible into hydrion concentration, and *vice versa*, hydrion determinations are always in terms of normality, for *p*H signifies the minus logarithm of normality. These points will become apparent in future exercises.

Preparation of Standard Solutions.—For purposes of titration to be given in the following pages it is essential to use standard solutions of known normality. The principles of the composition of such fluids will be given now as practical exercises:

Exercise 5.—*Standardization of Chemical Glassware.*—While at the present time commercial glassware has reached a more dependable stage than heretofore, it is never safe to assume that a pipette or burette is correctly calibrated.

(I) *Calibration of a 5-ml. Pipette.*—Secure, from the store-room, some slips of gummed paper. Warm some cleaning solution[1] in a

Fig. 43.—Wash-bottle.

small casserole or beaker and pour it through the mouth-piece of the pipette (never suck up such a solution), drop by drop, rotating the pipette and inclining it so that all parts of the interior surface are brought into contact with the solution. Hold the mouth-piece of the pipette against the palm of your hand with the tip directed upward at an angle of about 45° and hold your hand beneath running water from a tap; this will force the water upward through the pipette and insure that all portions of the inner surface come into contact with the cleansing fluid. After about a minute's washing, rinse with distilled water from your wash-bottle, remove as much adhering water as possible by shaking the pipette, and then remove the water by sucking up through it, first, ethanol and then ether in small amounts from a beaker. Finally, insert the tip of the pipette into a piece of rubber tubing attached to the side-tube of a filter-pump (S, Fig. 241) and pass air through the pipette for about five minutes. The instrument

[1] Appendix.

is now thoroughly clean. By means of a piece of filter-paper remove all oil from the hands that accumulates on the exterior of the pipette while handling, so that the tube is bright and clean and quite transparent. Now carry the tube, along with the pieces of gummed paper, two 50 ml. beakers, and your wash-bottle to the balance room, where you are assigned a balance. First adjust the balance: See that the rider has been removed from the graduated beam, that the pans are clean, and then note the swing of the pans; they should not

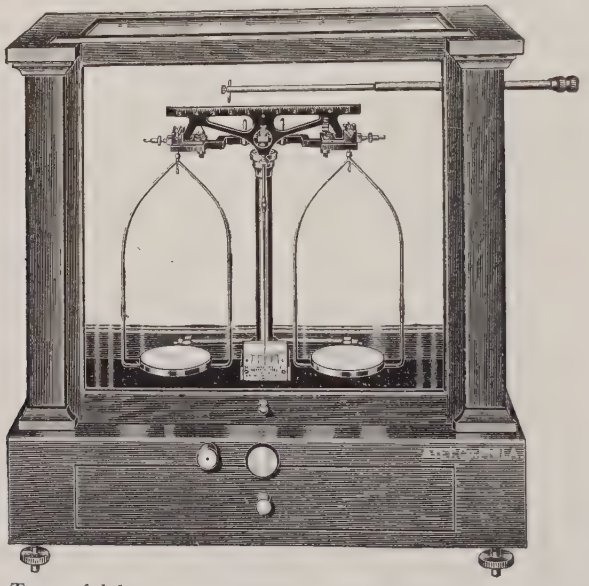


Fig. 44.—Type of laboratory balance. Great care must be exercised in using the balance if exact results are sought. Load the balance only while the knife-edges are not in contact. For weighings below 1 gram use the plunger at the left of the central milled head for releasing the pans.

cause the indicator to vary more than 12 spaces on the graduated scale as the beam rocks. If anything is wrong with the instrument, call the attention of the instructor to it. Do not attempt adjustments yourself. After bringing the instrument to rest, place a beaker on each pan and adjust the sweep of the pointer to equilibrium by adding a drop or more of water from your wash-bottle to the lighter beaker. Remove the delivery tube from your wash-bottle, insert the pipette, and draw up a column of water past the mark by about 2 cm. Place the tip of your index-finger over the upper end of the pipette to prevent the escape of the water, remove the pipette, dry the exterior carefully with a piece of filter-paper, and then adjust the

meniscus. Hold the tip of the instrument over one of the beakers on one of the balance pans; permit the water contained in the pipette to flow into the beaker until the contents are quite removed, the last portions being removed by capillary action as the tip of the pipette is held against the side of the beaker. Let it remain ten seconds; there will be left a small amount of water in the extreme tip[1] that is

Fig. 45.—Rider for analytical balance. Five mg. riders are used with the balance (Fig. 44).

Fig. 46.—Box of gravimetric, analytical weights for use with the balance (Fig. 44). Weights must be handled only with care, with the tweezers provided with each box, and never permitted to touch the hand or any corrosive chemical. The weights shown above are from 0.500 to 0.001 g. For the larger weights see Fig. 47.

never removed, since the pipette is graduated—not to contain 5 mls., but to deliver that amount. Now proceed to weigh the water delivered from the pipette by adding weights until the balance is again in equilibrium. Compare the weights with those given in the table[2] which gives the weight of pure water at different temperatures

Fig. 47.—Analytical balance weights. Compare Fig. 48 for weights less than 1 gram.

Fig. 48.—Set of weights for use with the analytical balance, from 1 to 500 mgs. (0.001–0.500 g.). These weights must be returned to their proper places in the box (Fig. 46) after use. They are to be handled with forceps.

for volume like 1 ml. If the pipette needs adjustment,[3] make it in this manner: Balance the instrument and then lay weights upon one pan equal to the weight called for, for a volume of water at the temperature of the experiment, as indicated by the table. Fill the pipette again, as in the beginning of the exercise, but adjust the

[1] The description is for a "delivery" pipette; a "tip-off" or "contain" pipette must have the last drops removed by the method given in the Appendix.

[2] Appendix.

[3] It is well to make three determinations and take the average of the three.

meniscus slightly above or below the mark on the pipette according to the error. Now let the water flow from the pipette into the lighter beaker, drop by drop, until the instrument is in equilibrium; stop the flow immediately. When the proper point is reached, after repeated trials, place a piece of moistened gummed paper exactly opposite the meniscus of the column of water in the pipette. This is the correct mark for the pipette. By means of a file,[1] etch a shallow ring around the tube of the pipette to indicate the new marking. Use this pipette for other calibrations mentioned below.

Calibration of a 250-*ml. Volumetric Flask.*—Proceed in the manner described above for standardizing a pipette, making necessary modifications for the type of glassware in this case.

Fig. 49.—Volumetric flask. In accurate work the temperature of the fluid must be 20° C.

Calibration of a 25-*ml. Volumetric Cylinder.*—Clean and dry the cylinder as directed above for the pipette. Fill the calibrated 5-ml. pipette with distilled water, noting temperature. Adjust the meniscus to the corrected mark. Empty the pipette into the cylinder as described for the pipette and note the position of the meniscus in the cylinder. Note any discrepancies between the present calibration and that done by the manufacturers of the cylinder and note, in your note-book, the adjustments necessary for the different 5 ml. markings.

Calibration of the 1-*ml. Ostwald-Folin Pipette.*—This should be done with the greatest care: Proceed exactly as described for the 5-ml.

[1] After a duplicate determination for the sake of accuracy.

pipette, but using the quantities appropriate for the smaller pipette, as given in the table in the Appendix. Recall that this form of pipette is a *contain* pipette and the contents as a whole must be used; see Appendix.

Calibration of a 25-ml. Burette.—This may be done by filling the cleaned burette to the 25 ml. mark toward the lower portion of the instrument with distilled water and delivering 5-ml. portions of water at known temperature to the burette from the calibrated 5-ml. pipette, noting in your book the discrepancies observed. Or, the burette may be filled to the zero mark at the top of the instrument and weighing successive 5-ml. volumes as described for the 5-ml. pipette calibration.

Fig. 50.—Arrangement for burettes without glass stop-cocks. In place of the metal clamp a glass bead may be inserted into the rubber tubing, which, being pinched, permits the flow of liquid from the burette.

Procedure for Making Standard Solutions.—Place about 25 gs. of sodium hydrogen carbonate, $NaHCO_3$, in an evaporating dish and bring the temperature to 180° C. on a sand-bath, or, preferably, in a thermostat automatically held at that temperature.[1] Leave for half an hour or longer, remove the dish by means of crucible tongs, and transfer the contents to a small, clean, warmed bottle which can be tightly stoppered. Let the contents cool and then place the unstoppered bottle in your desiccator[2] over H_2SO_4. Leave until the following period, giving your attention now to the making of standard acid (Exercise 6). When you are able to return to the preparation of the alkali, proceed as follows: From the bottle in the

[1] The reaction involved is as follows: $2NaHCO_3 = Na_2CO_3 + H_2CO_3 \uparrow$ (the arrow means that H_2CO_3 passes off as a gas).

[2] The Hempel form of desiccator, in which the acid is contained in the rim of the cover of the desiccator is preferable, because of water-vapor rises. The best way to make an H_2SO_4 desiccator for ordinary use is to soak lumps of pumice about the size of cranberries with pure concentrated H_2SO_4 and lay them in the bottom of the desiccator. The dish containing the reagent undergoing desiccation is supported upon short pieces of large size glass tubing laid horizontally.

desiccator weigh out half-gram lots of the sodium carbonate[1] formed from the bicarbonate, according to the following directions: Weigh the loosely stoppered bottle to the third place of decimals accurately and enter the figures in your note-book. Remove the stopper by means of crucible tongs and with a clean spatula or knife-blade, remove to a small Erlenmeyer flask, without spilling, about a half spoonful of the powder. Stopper the bottle and the flask and then reweigh the bottle as before. Subtract this weight from the previous weight and label the Erlenmeyer flask with glass-marking pencil, indicating the weight of the Na_2CO_3 within it. Repeat this procedure three times. You have now obtained three accurately weighed

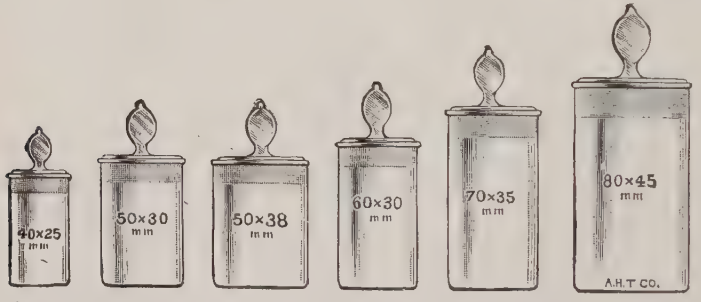


Fig. 51.—Weighing bottles of various capacities. For careful analysis the substance must be placed in the bottle of suitable size, the bottle weighed, and then about the required amount is removed; a second weighing shows the amount removed.

samples of alkali. Add to each a little distilled water and dissolve the powder. Add one drop of indicator (methyl orange; brom-phenol-blue; methyl red, or other good indicator, excepting phenolphthalein) to each flask. From a burette holding some of the standard acid, made according to the directions given in the following Exercise, run in, carefully, acid until the indicator is seen to exhibit a change in color. Stop the instant any change is observed and arrange a control specimen by adding a drop of indicator to a little distilled water in a test-tube or Erlenmeyer flask, and then add a drop of the standard acid from the stock bottle. Now, with this control giving the color to which titration is to be carried, before you, proceed to add acid drop by drop from the burette until the control is reached. Take the reading, enter it in your book, and also pencil it on the Erlenmeyer flask. Repeat with the second and third Erlenmeyer flasks in similar manner. Now with these figures, determine the normality of the acid: If the acid were exactly decinormal, each ml. would contain

[1] See the reaction given in the footnote, page 93.

0.0049 g.[1] of H_2SO_4 and n mls. would therefore contain 0.0049 n. gs. Therefore:

Sought..... 0.0049 n. gs. H_2SO_4

The amount found is as follows: The fundamental equation is:

$$H_2SO_4 + Na_2CO_3 = H_2CO_3 + Na_2SO_4.$$

$$\frac{H_2SO_4}{Na_2CO_3} = \frac{98.076 \text{ (mol. wt. of } H_2SO_4)}{106.000 \text{ (mol. wt. of } Na_2CO_3)}$$

The amount of Na_2CO_3 taken for the first titration is y gs., which requires for neutralization by the H_2SO_4:

$$\frac{98.076}{106.000} = \frac{x}{y}$$

$$x = 0.925\ y.$$

Therefore:

Found: 0.925 y gs. H_2SO_4.

The normality is the number of times the amount found must be increased or decreased to represent exactly normal H_2SO_4. Hence:

When "found" must be $\begin{matrix}\text{increased}\\ \text{decreased}\end{matrix}$ to equal "sought," $\frac{0.0049n}{0.925\ y}$ is the normality. Take the average of the three figures for normality as the strength of the standard acid.

With this standard acid prepare a standard decinormal solution of sodium hydroxid, NaOH, from the concentrated, 50 per cent. solution of sodium hydroxid as follows: By titrating against the standard acid solution, determine the normality of the hydroxid solution, using exactly one ml. of the acid. By proper dilution, adjust the normality of the alkali solution to decinormality.[2]

Method of Obtaining Standard Acid Solutions from Constant Boiling Hydrochloric Acid.[3]—Dilute desk reagent concentrated HCl until the hydrometer stands at 1.100. Place about 350 mls. in a distilling flask (500 ml.) and connect the flask with an Allihn condenser (Fig. 249). Attach a second distilling flask by means of a rubber stopper to the receiving portion (lower) of the condenser and lead a rubber tube from the side neck of the flask to beneath the

[1] Molecular weight of H_2SO_4 = 98.076. A normal solution contains one-half that amount, because of double hydrogen equivalent and a decinormal solution contains 0.1 of that amount of H_2SO_4, or 4.9038 gs., per liter of solution, each ml. of which contains 0.0049 g.

[2] If the normality of an approximately decinormal NaOH solution is exactly known, it is not necessary to make it exactly decinormal, but it is frequently convenient to have an exactly decinormal standard solution of both acid and base.

[3] See Hulett, G. A. and Bonner, W. D. Jour. Amer. Chem. Soc., vol. 31, page 390, 1909; also Bonner, W. D. and Branting, B. F. Jour. Amer. Chem. Soc., vol. 48, page 3093, 1926.

surface of water in a beaker; this precaution is to guard against the escape of uncondensed HCl fumes. Boil. When 200 mls. of distillate have distilled, remove the collecting flask, empty it, discarding the distillate and replace the flask on the condenser. Collect all but about 50 mls. of the remaining HCl solution. Read the barometer and enter the following table:

Bar.	HCl gs. per 100 mls.	To make one liter of normal HCl dilute.
760	20.242	180.155 gs.
750	20.266	179.945
740	20.290	179.730
730	20.314	179.515 to 1000 mls. H_2O

EXERCISE 6.—*Preparation of Approximately Decinormal Sulphuric Acid Solution.*—The commercial concentrated sulphuric acid of specific gravity 1.84 is about twenty times normal, because it is about 98 per cent. pure H_2SO_4, that is, 98 gs. H_2SO_4 per 100 gs. of "concentrated" solution. A decinormal sulphuric acid solution contains 4.9 gs. H_2SO_4 per liter.[1] Therefore 5 gs., or 3.0 mls., of the concentrated solution from the desk must be diluted to one liter to make a decinormal solution. Dilute to the mark with distilled water;[2] this makes a solution approximately decinormal.

SUMMARY

(1) Man's present organism, chemically as well as morphologically, is the result of development through long ages.

(2) About one-fifth of the total number of known elements are incorporated into the human body.

(3) While many of these elements are indispensable to the composition and maintenance of the body, hydrogen is of especial importance owing to its relation to the reaction of the tissues and fluids of the body. It is responsible for their acidity.

(4) A mechanism exists in man for the critical regulation of the reaction of the body, which is maintained at practical neutrality notwithstanding the great amount of acid produced in the tissues.

(5) Methods are given for determining both the acidity and the reserve basicity which regulates the reaction of the body. These

[1] Page 38.

[2] Throughout the work the temperature must be kept at about 20° C. (room temperature). The volumetric flasks are, as a rule, calibrated for 20° C. or else 15° C., or 25° C. The accuracy demanded by the present work will be met by maintaining the temperature at 20° C.

methods depend upon the determination of momentary acidity, due to the amount of hydrion per volume or weight of fluid or tissue.

(6) The method of titration, which determines the normality of a solution when dissociation of the acid-producing substances is 100 per cent., is of only limited use in biochemistry, since the critical factor ordinarily is the amount of hydrion free to manifest itself under the conditions existing at the time the determination is made.

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CHAPTER II

THE PROMOTERS OF CHEMICAL ACTION. THE ENZYMES[1]

"Deus magnus in magnis, maximus in minimis."—*St. Francis of Assisi.*

Peculiarities of Biochemical Reactions.— One of the most evident differences between animate and inanimate objects is the power of the living organism to perform quickly reactions which, outside it, require either prolonged intervals of time or special conditions, such as increased pressure and temperature. What power does the body possess by which it can perform these actions quickly at the ordinary temperatures and pressures to which it is subjected? The answer involves a study of the phenomena of surfaces and membranes which separate different parts of the organism. Each cell is separated from other cells by a membrane, and within each cell are separating surfaces, such as the nuclear membrane surrounding the nucleus. At the surface of the nucleus many important phenomena occur. It is there that the granules are first seen in a secreting gland-cell. Cartilage and bone first form at the boundary between nucleus and cytoplasm (Fig. 52). These are only a few of the phenomena of surfaces. At the present time such phenomena are largely explained on physical-chemical grounds, under the general category of capillary, or surface phenomena, but it is possible to show in certain cases that apparently purely physical processes are in reality special chemical phenomena, explicable by chemical equations. The number of such cases, however, is limited, and until more are discovered it is well to adhere to the physical conception. The only truly chemical processes of this nature that have been satisfactorily explained are those of three enzymes concerned with oxidation: (1) *thyroxin*,[2] the principle of the thyroid gland, insufficiency or absence of which leads to hypothyroidism;[3] (2) *glutathione*, found in various tissues;[4] (3)

[1] The term "promoter" has been used in technology to designate an agent which works with another substance in producing a certain reaction. Thus, in a process for synthetic ammonia, iron is used to hasten the process, but if tungsten is added also, the reaction is more complete. Iron is called the *catalyzer*, and tungsten the *promoter*. Here, however, we use the term synonymously with catalyzer.

[2] Page 99.

[3] See page 141 concerning Harington's investigations on thyroxin and the "enzyme" concept.

[4] Page 343. The "Bios" of Wildiers and of Eddy may be a fourth case, as Kendall suggested. (Page 560.) For an account of glutathione see Evans (page 26).

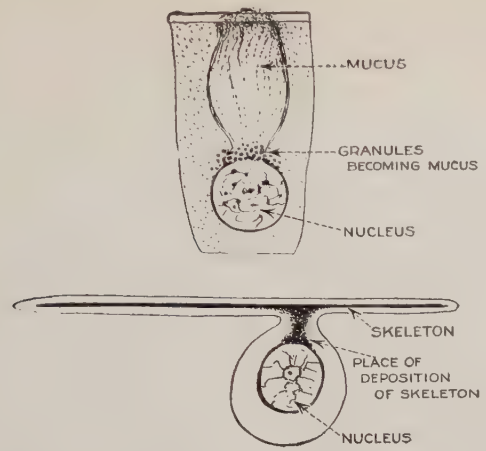


Fig. 52.—Illustrating the importance of the interface in the organism. Above, a gland cell, secreting mucus; the secretion arises at the interface between nucleus and cytoplasm. Below, a secreting skeletal cell, showing the origin of the skeletal substance in the region of the interface, the nuclear membrane. Such a cell is encountered in the spicule-forming cells of the sponge animal and, in a modified manner, in the human bone-forming cell.

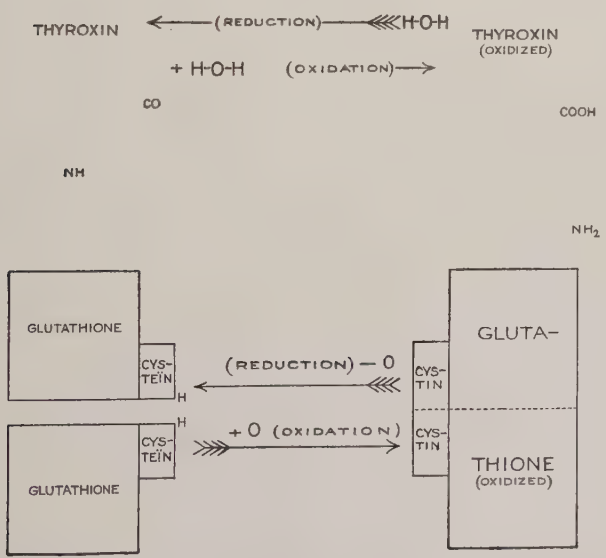


Fig. 53.—Illustration designed to show, diagrammatically, two of three demonstrable cases of enzyme action, involving an acceptance of oxygen and a release of oxygen. As a result the freed oxygen is available for oxidations in the body. A similar action is seen in the case of glutathione.

urease crystallized by Sumner and identified as a globulin.[1] We shall discuss these later.

Colloidal States of Matter.– When matter somewhat grosser than molecules exists in such a form that much surface is exposed in proportion to its bulk, that state is called colloidal.[2] The surface of a given sphere compared to its volume may be greatly increased by breaking it up into smaller bodies. Each time a sphere is divided (Fig. 54) two halves are formed and the line of cleavage between them adds to the surface of each half, the area circumscribed by the line A-B. The surface of two spheres are related by the squares of the radii, whereas the volumes are related by the cubes. Consequently, as the size (radius) of the sphere is reduced by cutting it into two portions, the volume is relatively more reduced than the

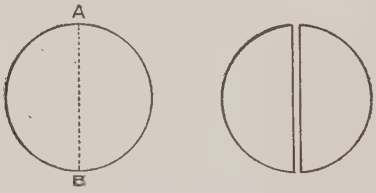


Fig. 54.—Illustrating the increase in surface as a sphere becomes divided. This is the principle of the action of bile in fat digestion.

surface. This principle is at the basis of the emulsification accompanying the digestion of fat.[3] The properties of a substance in finely divided state differ from those of the same substance in larger masses; or, as Ostwald[4] said: "Every structure assumes special properties and a special behavior when its particles are so small that they can no longer be recognized microscopically, while they are too large to be called molecules."

Enzymes Are Known to be Colloidal.—Enzyme action seems to resemble in many respects the behavior of matter in finely divided state, known as catalysis,[5] an example of which we shall now present:

Exercise 1.—Into 5 mls. of 3 per cent. hydrogen peroxid solution pour one volume of colloidal platinum solution made by the labora-

[1] Page 709.

[2] Greek *kolla*, glue, and *oidos*, like; so named by Thomas Graham, English physicist of the 19th century.

[3] Page 457.

[4] W. Ostwald, German chemist.

[5] Greek *kata*, down, and *lysis*, loosen; we should say "loosen up." For a discussion of the chemistry of enzyme action and related topics see Falk.

tory.[1] Note the evolution of gas according to the reaction:

$$\begin{array}{l} H{-}O{-}O{-}H \\ H{-}O{-}O{-}H \end{array} \xrightarrow{Pt} \begin{array}{l} H{-}O{-}H \\ H{-}O{-}H \end{array} + O_2.$$

Two hydrogen peroxids. Platinum. Two water and oxygen.

In the concentration given, the reaction is a bimolecular one, that is, two molecules are involved ($H_2O_2 + H_2O_2$). In dilute solutions, and in cases in which there is no oxygen acceptor, the reaction is unimolecular (one in which one molecule gives rise to two or more molecules).

The Surface Phenomenon of Platinum Black.—In the preparation of the platinum solution used in the Exercise just given a piece of platinum wire about 0.1 cm. radius was divided, electrically, into small particles, so small that they are invisible under the highest powers of the microscope. The platinum, considered as a sphere 0.1 cm. in radius, is broken into small spheres with a radius each of $\frac{1}{1{,}000{,}000}$ centimeter.[2] The surface of the platinum wire, considered as a sphere, is 0.628 sq. cms. The combined surface of the small platinum colloidal particles is over 100 *sq. meters*, that is, about 10,000,000 times greater than the original surface of the platinum. Figure 56 shows the relative sizes of particles exhibiting colloidal properties, compared with the proportions of some common microscopic objects, such as bacilli and blood-corpuscles. This extent of surface of the colloidal particles is responsible for the hastening of the reduction of hydrogen peroxid according to the equation given above.

Everyone knows that certain chemicals tend to become attached to surfaces. Water adheres to a pane of glass exposed to the rain. Iron adheres to porcelain of the bath-tubs. On the other hand, some substances will not adhere to surfaces: Mercury does not adhere to glass. It is known that the same chemical affinities which matter exhibits in chemical reactions are responsible for the adhesion of substances on surfaces. The question may be asked, What has this power of adsorption to do with hastening chemical reactions? Noyes[3] and Whitney[3] first showed and Brunner[3] corroborated the fact that at such surfaces the molecules of H_2O_2 exist in a saturated solution which permits a greater number of molecules to come into contact with the platinum. But what causes a reduction of

[1] The method is given in the Appendix.

[2] The unit of measurement of these small particles is the millimicron, one millionth of a millimeter and abbreviated $\mu\mu$. One μ is a thousandth of a millimeter.

[3] Noyes, A. A. (California Inst. Tech.), Whitney, W. R. (Gen. Elec. Co.), Brunner, E. (in Nernst's Laboratory, Berlin, Germany). Zeitschr. f. physik. Chem., vol. 23, page 689, 1897.

H_2O_2 in its saturated layer? It has been shown by Langmuir[1] that such adsorption involves the spread of the substance, or the tendency for it to spread over the colloidal particle in a very thin layer, frequently one molecule thick.[2] In this condition, there is an orientation of molecules as explained on page 115 and the reaction of H_2O_2 is, therefore a chemical process. Dust will produce the same result as platinum black, and for this reason the surgeon who uses H_2O_2 must prevent dust from falling into his opened bottle of peroxid.

The Surface Phenomenon of Gold Particles.—The Lange[3] test for cerebrospinal syphilis and other diseases depends upon changes in the color of colloidal gold preparations (Fig. 55). Certain abnormal constituents of the fluid precipitate the gold particles, and the color varies according to the number of particles left in suspension in the solution. The particles of gold are about $10\mu\mu$ in diameter. The abnormal substances must reach a certain concentration in the fluid before they will cause the precipitation of the gold particles.

Demonstration.—Colloidal gold is formed by reducing gold solutions by means of formaldehyde,[4] and the distribution of the gold particles throughout the solution causes the color. The particles have been shown by Scherrer, a German chemist to contain 380 atoms of Au. The character and degree of dispersion determine whether the solution can be used in the Lange test. Tabetic fluid causes the precipitation known as Group 1; cerebrospinal meningitis, Group 2; and paresis, Group 3.

Peptization.[5]—If chlorid, bromid, or iodid is added to the gold solution the solution becomes more stable. These substances form compounds with gold. On the other hand, the addition of fluorid to gold solutions causes the particles of gold to become precipitated and to mass together. The term "peptized" is used to indicate that a colloidal solution is relatively stable. Fluorid does not form a combination with gold, and therefore has no peptizing power. When peptization occurs, the colloidal particle assumes the electrical charge of the substance peptizing it. Chlorid, Cl^-, is negatively

[1] I. Langmuir, Chemist, General Electric Company, Schenectady, N. Y.

[2] Persons familiar with photography know that if a plate is in contact with the sides of the developing tray, that edge develops somewhat more rapidly than other parts.

[3] C. Lange, German physician, contemporary.

[4] The method for making the solution is given in the Appendix.

[5] The most satisfactory reference aside from original sources is the article of Thomas (A. W. Thomas, Associated Professor of Food Chemistry, Columbia University) in Bogue; see page 129.

Fig. 55.—Colloidal gold reactions. Application of colloidal chemistry. The upper set shows a reaction with the cerebrospinal fluid of a paretic ("paretic curve"); the lower set shows a reaction with the cerebrospinal fluid of a tabetic

charged and endows the gold with this charge. It is impossible to have only negative charges; positive charges must be present within the limits of the electrostatic distance.[1]

$0.1\mu\mu$	$1\mu\mu$	$10\mu\mu$	1μ	10μ	100μ	1 mm.
Diameter of H_2 mol.	Diameter of starch mol.	Diameter of gold suspension.	Diameter of clay suspension.	Maximum length of bacillus.	Limits of unaided vision.	

Diameter of ethanol molecule. Gold particles begin to settle.

At about 1μ particles begin to pass through ordinary qualitative filter-paper.

Brownian movement disappears.

At about $1\mu\mu$ colloidal phenomena begin to appear.

Chart showing relative sizes of submicroscopic, microscopic, and visible objects (unaided vision).

1μ (micron) = 0.001 mm. = 0.00,003,937 inch.
$1\mu\mu$ (milli-mu) = 0.000,001 mm. = 0.00,000,003,937 inch.

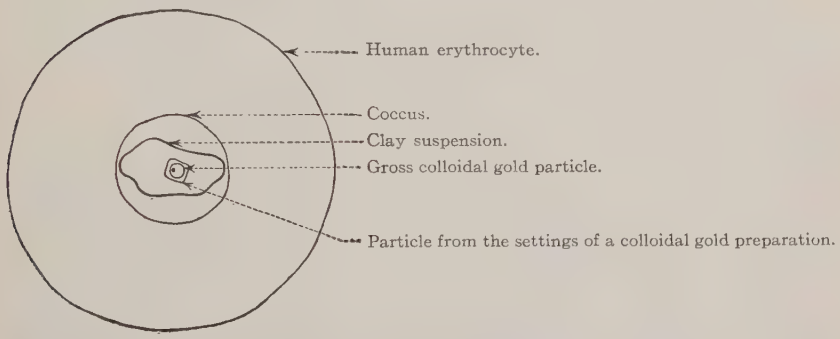


Fig. 56.—The diagram is designed to show the dimensions of particles in colloidal state. The dot at the center represents a hydrogen molecule, much too large.

Why the Suspended Particles Remain Suspended in Solution (Fig. 57).—The negative charges on the gold particles bring to themselves positive charges of the cation Na^+, if NaCl be added. Besides these cations, the charges of which balance the negative charges of the gold, there are the Na^+ cations ionized along with Cl^-. More Na^+ than Cl^- ions are present near the gold. The total number of ions near the gold will be greater than in the solution. This condition means that an electrical potential difference occurs between the gold particles and the solution, which tends to bring the ions together, but to cause repulsion of the gold particles. We can think, then, of gold particles being mutually repellant within the solution, much as a room-full of tennis balls bounding against each other.[2]

[1] The distance that charges may move apart from one another. The electrostatic distance varies with the kind of substance in which the ionization occurs, as well as with the substance itself.

[2] Concerning the charges on the gold particles, see Bogue, article by Wilson.

The Rate of Movement of the Particles.—If the colloidal gold particles could move as quickly as the ions, there would be no difference in distribution of ions on the particles and in the solution. However, one of the chief characteristics of all colloidal particles is the relatively slow movement. The movement of the particles may be seen under special forms of illumination.

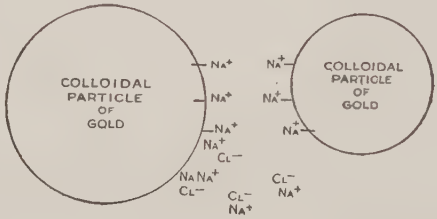


Fig. 57.—Diagram to show the cause of the suspension of colloidal particles in a solution. The particles of gold act as anions, holding the positively charged cations, Na^+, and since like charges repel, each particle repels all others. Note the potential difference between particles and solution: Two classes of cations are found near the gold, namely, (1) The Na^+ having the gold as anion and (2) the Na^+ having Cl^- as anion. There is a membrane potential difference (Donnan's[1] equilibrium).

The Ultramicroscope; *Demonstration.*—This instrument is a condenser placed beneath the stage of a microscope. The construction is shown in Figs. 58 and 59. All direct light is stopped by the diaphragm D, but light which comes from an angle passes across the stage of the microscope more or less horizontally. If there are particles on the slide, light is caught by them and reflected or refracted up the tube to the eye. The phenomenon is much like that of planets at night; we see them against a black background, the sky, because they reflect the light from the sun, which is hidden from us by the earth. The particles do not present definite discs, even under the highest powers of the microscope; the same is true of the planets seen through opera glasses.[2] It is the pencil of light coming from them which strikes the eye. The principle of the ultra-microscope may be shown by the Faraday-Tyndall phenomenon:

The Faraday-Tyndall Phenomenon;[3] *Demonstration* (Fig. 60).—Place three rectangular museum jars of clear glass upon the desk and to the first add distilled water that has just been distilled and is free from dust; to the second add colloidal gold solution, and to the third,

[1] See page 107, Donnan first described his theory of membrane equilibria to a physiological audience, London (England), Physiological Society, December, 1910.

[2] The analogy is perhaps better with the fixed stars which do not give a disk with the highest powers of the telescope.

[3] This exercise is frequently called the Tyndall phenomenon, but it was used by Michael Faraday at the Royal Institution, London, before Tyndall employed it.

add dialyzed iron solution.[1] Throw a beam of light from a small arc or concentrated filament electric lamp provided with a condensing lens[2] through the jars successively and note the intensity of the light-beam. Water, when distilled with the greatest care contains suspensions and there will always be some beam, but there will be great differences between the beam in the water and in the colloidal gold

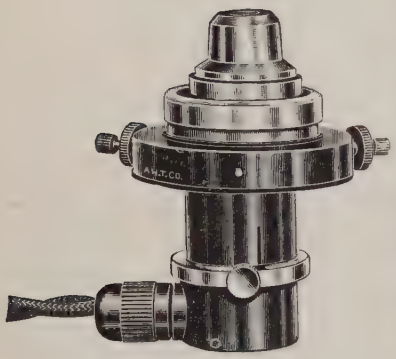


Fig. 58.—"Ultramicroscope." The apparatus fits beneath the stage of an ordinary microscope, replacing the condenser. The path of the light through the apparatus is shown in Fig. 59.

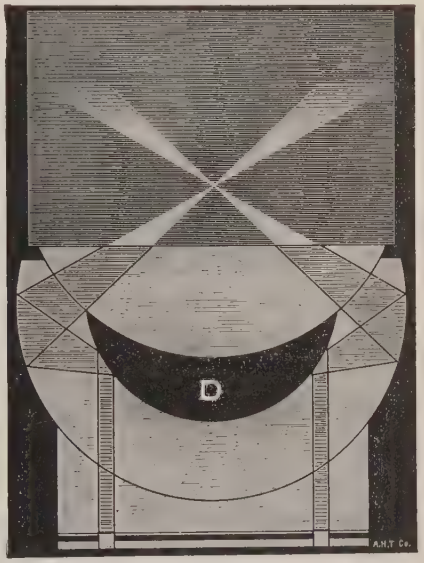


Fig. 59.—The path of rays in the "ultramicroscope." The colloidal substance is contained in the vessel in which the rays cross. The black disk D prevents light from passing directly to the eye. The light which reaches the eye is only that which is refracted by the colloidal particles.

and iron suspensions. Dilute the liquids and determine when the beam, characteristic of the solution, disappears.

All colloidal solutions show this phenomenon.

Brownian Movement.—Under the ultramicroscope the minute particles may be seen to dart here and there in zigzag paths. This motion was discovered by the British botanist, Brown, and has been named after him. It is due to the bombardment of the colloidal particles by molecules and ions. The movement of the ions and

[1] This is the pharmaceutical preparation known as Dialized Iron or Liq. Ferri Dialyzatus, consisting of ferric hydroxid solution dialyzed against distilled water with a small amount of ferric chlorid.

[2] The light rays should enter parallel.

molecules cannot be seen, but that of the colloidal particles (many times larger), as they are pushed about by the smaller bodies, is visible.

The Colloidal Nature of the Living Substance.—The typical food substances—starches, fats, and meat—are colloidal in nature. They have large molecules, which behave much as the gold particles.

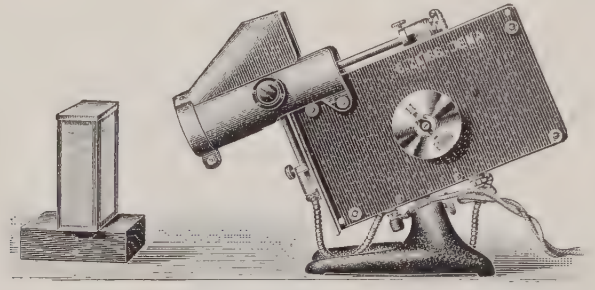


Fig. 60.—The Faraday-Tyndall phenomenon. The jar contains the colloidal suspension. Light from the arc passes through the suspension, causing a beam of light produced by reflection and refraction by the colloidal particles.

Moreover, they are limited by membranes which prevent them from moving freely. Like the gold particles, these food substances hold ions and restrain them from moving away; consequently, there is the same unequal distribution of ions around the molecules, and the cause of their being in suspension in solution is the same.

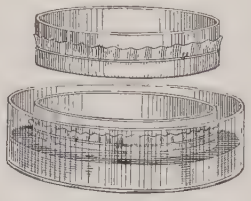


Fig. 61.—Graham's apparatus for the application of dialysis. Graham's experiments laid the foundation for the modern theories of the structure of colloidal states, of protoplasm, etc. (From Holland, Medical Chemistry and Toxicology.)

Exercise 2.—*Dialysis of Colloidal Solutions.*—The preparation of the dialyzing membranes is described in the Appendix.

Into sac A, suspended in a beaker of distilled water, place 25 mls. of a 1 per cent. dialyzed iron solution.

Into sac B, similarly suspended, pipette 25 mls. of egg-white solution.

Into sac C, similarly suspended, place 25 mls. of a 1 per cent. NaCl solution.

Into sac D, similarly suspended, pipette 25 mls. of a 1 per cent. solution of mastic.[1]

Into sac E, suspended as before, place 25 mls. of a 5 per cent. tannic acid solution in water.

Into F place 25 mls. of a 5 per cent. alcoholic solution of tannic acid.

Set the beakers containing your preparations in your locker, and leave until the following period. Then analyze the dialyzates for evidence of the dialysis of the special agent introduced into the sacs, as follows:

Sac A: Tests for the presence of ferric iron: (I) Add a drop of potassium ferrocyanid; a positive test for ferric iron is indicated by the appearance of a blue color (Prussian blue). (II) Add a drop of potassium thiocyanate; red ferric thiocyanate is formed.

Sac B: Test for egg-white; see page 224, Exercise 2, as far as "Repeat, etc."

Sac C: Test for chlorion; see page 359.

Sac D: Test for mastic; mastic is a gum insoluble in water. Select two test-tubes as nearly alike as possible. Fill one with pure, freshly distilled water. Fill the other to the same height with the dialyzate. Compare the appearance of the two solutions, looking down the tubes. If the mastic suspension has passed into the dialyzate, it will exhibit a cloudiness compared with water. A confirmatory test may be made; fill a third tube ⅓ with 95 per cent. ethanol and add to the alcohol sufficient dialyzate to bring the volume to that of the two first tubes. Does the precipitate observed in the second tube disappear on mixing the contents of the third tube?

Sac E: Tannic acid has the property of precipitating egg-white from solution; utilizing this principle, place 10 mls. of the dialyzate in a test-tube and add, drop by drop, pure egg-white,[2] noting any coagulation.

Sac F: Repeat the procedure just given for Sac E. The colloidal nature of the substances used in Sacs A, B, D, and E is evident.

The Donnan Equilibrium.—Donnan[3] showed that there is an unequal distribution of charges wherever one part of the system prevents the free movement of molecules and ions. We have illustrated this theory in the discussion of the cause of the suspension of gold particles in colloidal solution. An illustration is also afforded by the dialyzed iron colloidal solution: 5 per cent. ferric chlorid is held by the ferric hydroxid,[4] which would otherwise dialyze. Loeb[5] applied this theory to the behavior of such substances as gelatin and other protids. These compounds, also starch and fat, do not diffuse through membranes like collodion, parchment, and animal mem-

[1] See Appendix for method of preparation.

[2] This egg-white must have been dissolved in water and filtered. It should be a concentrated solution, about 5 or 10 per cent.

[3] F. G. Donnan, Professor of Physical and Inorganic Chemistry, University College, London. An explanation of the theory of Donnan is given by Bayliss, by Loeb, and by Donnan in his review in Chemical Reviews, vol. i, p. 73, 1924. See page 104.

[4] The ferric hydroxid is the true colloidal solution, ferric chlorid being a crystalloid.

[5] J. Loeb, Rockefeller Institute, died 1924 at Hamilton, Bermuda.

branes, but nevertheless hold certain ions captive; the result is the same as that described for gold particles. Gelatin is an amphoteric[1] compound; that is, it has the properties both of an acid and of a base. As base, it forms a salt with anions, such as Cl^-; and as an acid, it forms a salt with cations, such as Na^+. Gelatin also receives anions and cations, which are equally distributed on either side of a membrane separating gelatin from the medium in which it exists. Hence, as in the case of the gold particles, we have a preponderance of ions in the gelatin because we find there (1) ions chemically bound by the gelatin, and (2) ions equally distributed throughout the whole solution. The result for gelatin, then, is:

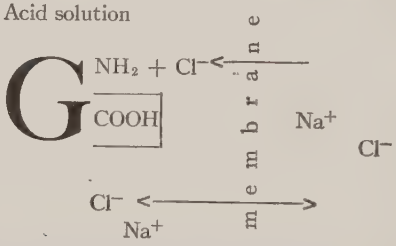


Diagram showing the unequal distribution of ions on two sides of a membrane separating gelatin from a salt solution. The gelatin acts as a cation in acid solution, and as an anion in alkaline solution. Note that there are more Cl^- anions on the gelatin side than on the salt-solution side of the membrane.

As a consequence of the unequal distribution of ions, gelatin assumes the characteristics known as colloidal; the like signs of the ions concerned repel one another and also the gelatin bearing them.

Cataphoresis.[2]—Colloidal particles bear characteristic charges of electricity. Thus, colloidal iron (page 107) is electropositive and is precipitated by basic substances like NH_3, NaOH, etc. On the other hand, mastic (page 107) is electronegative and is precipitated by HCl and by acetic acid. When free to move in an electric field, as in the following demonstration of cataphoresis, these electrical relation become manifest.

Demonstration.—The Y-tube connects with a rubber connection leading from the bottom A, whereby the solution in the leveling bulb may be led into the two portions of the Y. The tube is clamped by means of a screw-clamp after the liquid in the tube has reached the turn of the limbs in Y. A solution of zinc sulphate is now poured into each of the limbs of the Y, carefully, to avoid mixing the two liquids, $ZnSO_4$ and the gelatin solution below. Electrodes of zinc

[1] Greek *amphi*, both, and *terma*, term.

[2] Greek *kata*, down, and *phoreo*, carry.

are lowered into each of the arms of the Y and then the gelatin solution is carefully added from below by gradually raising the leveling bulb after opening the clamp. When the solutions have filled the arms of the Y, clamp the screw-clamp and open the electric circuit, which should consist of about half the voltage of the ordinary house current (110 volts), that is, about 50 volts and of 6 amperes. After half an hour the presence of gelatin in each arm is tested.[1] If the gelatin were less acid than *p*H 4.6, it will be found on the anode arm of the cataphoresis apparatus, while if the solution were more acid than *p*H 4.6, the gelatin will be found in the cathodal arm (the −

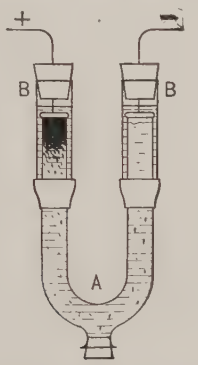


Fig. 62.—Apparatus for cataphoresis of colloidal solutions. The current is sent by way of the electrodes passed through the stoppers *B* and *B* into the fluid lying beneath them; this fluid is composed of molecules and ions similar to the composition of the electrode. Gelatin or other means is employed to separate this fluid from the unknown in the "U" tube. If the colloidal substance is positively charged, it migrates to the negative pole and vice versa. The figure shows a collection of anions at the positive pole. (Printed by permission from "General Chemistry" by H. G. Deming, published by John Wiley & Sons, Inc.)

arm). The explanation is that if the solution is more acid than *p*H 4.6, the gelatin is positively charged and moves to the opposite pole, the cathode (−), and if it is less acid, it moves to the anode (+). The reaction *p*H 4.6 is the iso-electric point at which gelatin moves neither toward one pole nor the other, being electrically neutral.

The Iso-electric Point.[2]—Several very important characteristics appear in colloidal solutions in the state of electrical equilibrium. In this state:

(1) Precipitation of the substance occurs.

(2) The substance is least soluble.

[1] The method is given on page 309, *f*, 1, 2, and 3.

[2] "I always feel inclined to leave the word 'point' to its proper use by mathematicians and geometers," R. B. Sosman, Geophysical Laboratory, Washington.

(3) The viscosity is least.
(4) Water is least absorbed.
(5) The particles tend to adhere to the greatest extent.
(6) Salt formation with acid or with alkali does not occur.

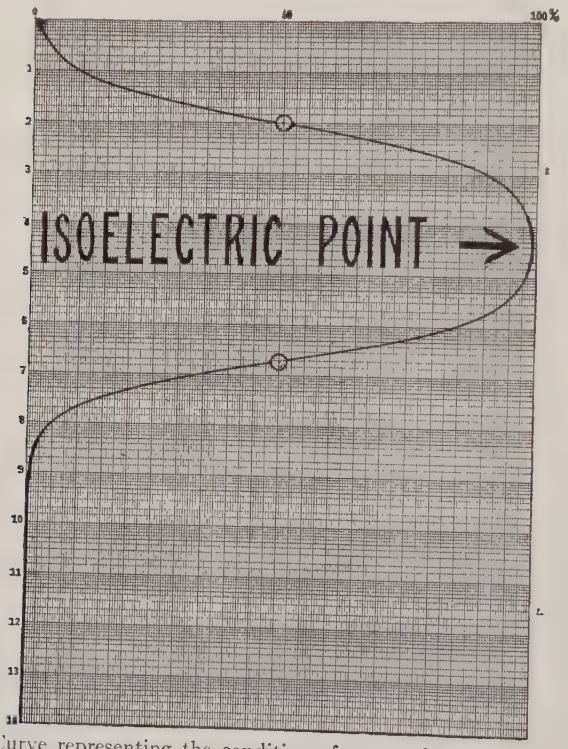


Fig. 63.—Curve representing the condition of an amphoteric electrolyte, such as gelatin, at the iso-electric point. At *p*H 4.6 neither the acid nor the basic portion of gelatin exerts itself. The curve is made on the Bovie direct reading potentiometer, Jour. Med. Research, vol. xxxiii, p. 295, 1915.

Exercise 3.—*The Determination of the Iso-electric Point of Pure Casein.*[1]—In a 50-ml. volumetric flask place 0.20 g. casein and dilute with 5 mls. 1 n sodium acetate solution,[2] 37° C. Add, while agitating the contents, distilled H_2O (37° C.) and continue to shake the flask until the solution becomes opalescent. Make up to the mark (50 mls.) with the warm distilled water. Arrange 9 test-tubes

[1] Pure casein can be obtained from the Harris Laboratories, Tuckahoe, New York. The method is that of Michaelis, L. and Pechstein, H., Biochem. Zeitschr., vol. 47, page 260, 1912 and revised in Michaelis Prac. Physical and Colloidal Chem. (see page 130).

[2] Thirteen and six-tenths gs. $CH_3.COO.Na.3H_2O$, crystalline, made up to 100 mls distilled H_2O.

of similar bore in a rack and add to each the amount of water, etc., indicated by the table (Mohr pipette):

Contents of tube number	1	2	3	4	5	6	7	8	9
Mls. distilled water	8.3	7.7	8.7	8.5	8.0	7.0	5.0	1.0	7.4
Mls. 0.01 n. CH_3COOH	0.6	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mls. 0.1 normal acid	0.0	0.0	0.2	0.5	1.0	2.0	4.0	8.0	0.0
Mls. normal acetic acid	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6

Then add to each tube 1 ml. of the casein solution; make the additions as rapidly as possible and, after stoppering the tube, invert it and shake it vigorously.

Tabulate in your note-book the following data:

Tube	1	2	3	4	5	6	7	8	9

On mixing contents:

"0," no change in appearance.

"x," precipitate.

"+" or "—," degree of opalescence in solution.

Tube No. 5 should show the greatest precipitation. The iso-electric point of pure casein is given in the Appendix, along with the same data for several biochemical substances. Employing the method for *p*H given on page 71, Ex. 4, using, however, methyl red as indicator and comparing the color with the laboratory standard.

The Iso-electric Point and Enzyme Action.—Digestion of meat substances, like steak, cheese, and similar compounds, requires coagulation or precipitation of the substance. In the case of milk, from which casein is obtained, if sodium bicarbonate is taken with it, casein does not become digested. The physician frequently administers sodium bicarbonate for various purposes, and milk is the universal food for the sick. Following ingestion of the two, flatulency often develops, owing to the fermentation of the milk-sugar, lactose. This action is enhanced by delay or retention of the undigested milk in the stomach. There are other instances in biochemistry of the inhibition of enzyme action when some agent prevents the precipitation of meat substances; an example is seen in the self-digestion (autolysis[1]) described on page 461, in which liver substance undergoes enzyme action at a certain degree of acidity. Bradley[2] finds a correspondence between the iso-electric point of certain substances and the point at which digestion appears:

[1] Greek, *autos*, self, and *lysis*, to loosen; also called maceration.

[2] Bradley, H. C., Professor of Physiological Chemistry, University of Wisconsin. See Physiol. Revs., vol. 2, p. 415, 1922 (Fig. 75).

TABLE SHOWING RELATION BETWEEN KNOWN ISO-ELECTRIC POINTS OF SUBSTANCES AND THE REACTION AT WHICH DIGESTION OCCURS (BRADLEY)

Substance.	Iso-electric point, *p*H.	Digestion begins at *p*H.
Egg-white (albumin)	4.8	5.0
" (globulin)	5.2	5.3
" (whole)	...	5.5–5.3
Blood serum (albumin)	4.7	5.0
" (globulin)	4.4	4.8
" (whole)	...	4.6
Hemp-seed substance (edestin)	6.9	7.0[1]

The Intermediate Compound in Enzyme Action.—While it has not been definitely demonstrated, it is probable that an enzyme attaches itself to the substance upon which it is acting and that a more or less definite compound is formed. This phenomenon may be illustrated in the following two experiments with inorganic catalyzers:

EXERCISE 4.—*The Catalytic Action of Water.*[2]—Place two or three pieces of granular zinc in a test-tube and overlay the zinc with concentrated H_2SO_4. Aside from the enmeshed air in the interstices of the granules of the zinc there is no evidence of gas formation which might be expected from the equation:

$$Zn + H_2SO_4 = ZnSO_4 + H_2.$$

Now add 1 drop of water and watch for an evolution of gas. Here the reacting substances are:

$$Zn^{++} + 4H^+ + 2OH^- + SO_4^=$$

and to explain the observed effects of water, it is necessary to assume that there is a temporary addition of ions to the zinc, perhaps as $Zn(OH)_2$. Afterward, by rearrangement, $ZnSO_4$ is formed which indicates the previous formation of an intermediate compound with the ions of water. Completing the equation:

$$Zn^{++} + H^+ + H^+ + 2OH^- + H^+ + H^+ + SO_4^= = H_2 + Zn(OH)_2 + H_2SO_4;$$
$$Zn(OH)_2 + 4H^+ + SO_4^=, = ZnSO_4 + 2H_2O + H_2.$$

Water is a catalyzer and appears at the end of the reaction in the same amounts as those at the beginning of the reaction.

EXERCISE 5.—*The Catalyzer Appears at the End of the Reaction.*—In the Kjeldahl reaction (page 113) the cupric sulphate appears at the end of the reaction and is indicated by its color. Place 1 ml. concentrated sulphuric acid in a test-tube and add 1 ml. of a 1 per cent. egg-white solution. To a second test-tube, add 1 ml. concentrated sulphuric acid, 1 ml. 1 per cent. egg-white solution, and one drop of

[1] Approximate.

[2] For a critical review of this subject see Rice, F. O. article in Taylor (see page 97), vol. 2, page 918.

5 per cent. cupric sulphate. Arrange the tubes so that they may be heated as nearly alike as possible. Note the time of the appearance of a clear yellowish-green color after the disappearance of the brown color due to the charring of the substance in the strong acid. The tube containing the cupric sulphate clears more quickly than the other tube and the clear green color of cupric sulphate remains, showing that it is a catalyzer, appearing at the end of the reaction. The reaction may be written:

$$2R.NH_2 + Cu^{++} + SO_4^{=} + 6H^{+} + SO_4^{=} = (NH_4)_2SO_4 + CuSO_4 + H_2,$$

the R being a radicle attached to the NH_2 group in the egg-white and the H_2 being used in the transformation of the R.

EXERCISE 6.—*A Typical Enzymatic Reaction.*—In your test-tube rack, set up five test-tubes of dimension 150 × 15 mm.

Into each place 5 mls. urea solution from the side-rack and 1.0 ml. urease solution prepared by the instructor according to Folin (Appendix). Then:

Tube A.—Leave at room T° C. 15 mins.

Tube B.—Place tube in boiling water-bath for two minutes and then return to your rack.

Tube C.—Add 2 drops of buffer solution for urea from side shelf (see page 711); leave as in A.

Tube D.—Add 2 drops of the buffer solution made by the instructor in a manner identical with that you used in Exercise 3, page 66, tube (pH = 5.28). Leave as in A.

Tube E.—Repeat D, using buffer solution of pH = 8.04 (like Exercise 3 page 66, tube 12). Leave as in A.

Control tube:—5 mls. of urea solution, alone. Leave as in A.

Make the additions as rapidly as possible to insure uniform time of action of the enzyme. At the end of 15 minutes, stop the action by immersing all the tubes in the boiling water-bath. Consult page 710 for the chemistry of urease action; note that ammonia forms when urease acts upon urea. Test for ammonia as follows:

(1) Moisten a piece of red litmus paper and secure it over the end of a test-tube containing 2 mls. of the solution from tube A. Boil the contents of the tube and note the appearance of blue color in the paper. Repeat with each tube. In your note-book indicate by + and − the tubes giving positive or negative tests.

(2) Dilute 1 ml. of each solution to 100 mls. In a series of test tubes of uniform bore place 1 ml. from each of the diluted urea

solutions, add 9 mls. of distilled water, mix and add 1 ml. of Nessler's solution (page 710). Arrange the tubes in the order of depth of color. Which solution yielded most NH_3?

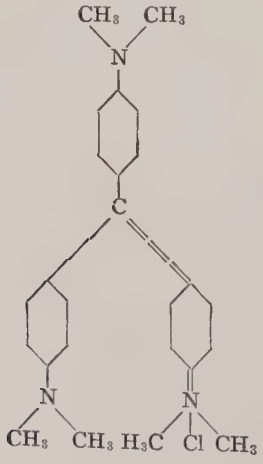
This experiment is of especial interest inasmuch as we are dealing with an enzyme that has been crystallized (page 709). We have introduced the subject of enzymes (page 100) as substances in colloidal state. In its natural form urease is colloidal; it will not diffuse through a membrane. The general principle that colloidal and crystalloidal states are possible in all substances is emphasized here. It was shown by Armstrong and Horton and by Van Slyke that urease action takes place at the surface of the colloidal particles.

Exercise 7.—*Adsorption.*—To a dilute solution of crystal-violet[1] in a test-tube add a knife-point of charcoal. Roll the tube between the palms in order to mix the charcoal and the dye. Filter. The filtrate should be colorless. If it is not, add charcoal to the filtrate and filter again. Suspend the charcoal in warm water and add aceton; the dye is dissolved from the charcoal. This principle has been used in the preparation of insulin;[2] here benzoic acid is used to displace the insulin from the charcoal. The quantitative relation, known as Freundlich's adsorption equation, giving the amount of carbon, M, necessary to decolorize X grams of color, leaving C units of color in the solution is:

$$\frac{X}{M} = K \times C^{\frac{1}{n}}$$

K and $\left(\frac{1}{n}\right)$ are constants for different kinds of charcoal.

[1] The formula is:



[2] Page 709.

The relation, then, between charcoal and the amount of dye is a quantitative one.

The Mechanism of Adsorption.—Adsorption is a surface phenomenon. We have shown that chemical reaction occurs between substances which cling to a surface and the substance adhered to. A substance which accumulates on the surface of a liquid lowers the energy of the surface (electrical, or surface energy-tension). Conversely, a substance which reduces surface energy tends to accumulate on the surface. These two facts constitute the Law of Willard Gibbs.[1] The importance of the subject for practical medicine is

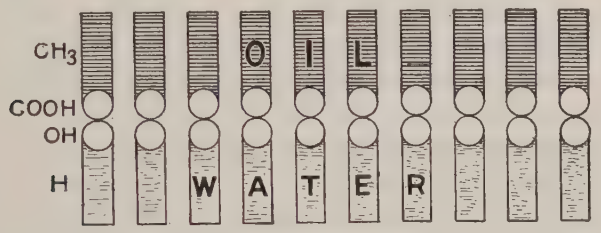


Fig. 64.—To illustrate the principle of surface tension. A fatty-acid, CH_3 . . . COOH, is dropped on water. The molecules become polarized or elongated, with the electrons of the atoms collected more towards one end (negative) and the nuclei (positive) towards the other. The active poles are towards each other, "like with like," that is, OH with OH in COOH.

witnessed by the attempts that have been made to block the destruction of blood and bile-pigments by causing the "blocking" of the reticulo-endothelium system.[2]

Surface Tension.—There is a difference in molecular arrangement on the surface and in the solution.[3] On the surface the molecules are oriented,[4] while in the solution they bear no definite arrangement

[1] J. Willard Gibbs, Mathematical Physicist, Yale University, deceased. His work laid the foundation of much of our knowledge of solutions. See page 117 for practical exercise.

[2] See Aschoff, L. (Freiburg, i. B., Germany). Lectures on Pathology in the United States, 1924. New York, P. P. Hoeber, 1924, p. 22.

[3] Van der Waals (Dutch physicist, 1873) in deriving an expression to embody the laws of Boyle and of Charles (page 79) for liquids and to account for derivations from calculated values, found it necessary to embody a factor to describe the different molecular behavior at the surface and within the solution; it is a in the formula

$$NRT = \left(P + \frac{a}{v^2}\right)(v - b),$$

in which NRT is evaluated on page 60, P and V likewise, b the volume actually occupied by the molecules and a the surface tension correction.

[4] Literally "turned toward the East," a saying taken from the religious habit of the Mohammedan, who prays toward Mecca. The expression means that there is a regular arrangement of the molecules.

toward one another.[1] On the surface of water the active portions of molecules are turned toward the surface. The active groups of the molecule are known as the polar groups. Oil is used by vessels at sea to reduce the tension on the surface; this permits the waves to pass the vessel without breaking. The waves break and fall with great force upon the ship if the surface of the sea retards the wave impulse. It is customary to represent the molecules of the surface by the character Ŭ, the polar or asymmetrical group being represented by the circle. In the case of fatty acid, CH_3 . . . COOH, acting to reduce the surface tension of oil,[2] we have the diagram (Fig. 64) to show orientation on surface of oil.

If it is true that the polar groups are directed toward the water and the methyl groups are freely exposed to the surface, the surface energy of water covered by various substances, like acids, alcohols, etc., in which the terminal groups are CH_3, should be the same. This is known to be true. The hydrocarbons arrange themselves on water with the CH_3 groups extending outward. The surface energy of the hydrocarbon-water interface[3] is similar to that of $CH_3OH - C_2H_5OH$-water interface, for the CH_3 groups of the alcohols lie exposed to the surface.[4]

Exercise 7.—*Hay's*[5] *Test for the Presence of Bile Salts.*—Arrange two small beakers before you. Place 100 mls. distilled water in each. To beaker A add a small amount of bile. To both beakers add sprinklings of flowers-of-sulphur. Note that in A the sulphur particles drop through the surface and sift slowly to the bottom, while in B the flowers remain on the surface. This test is used clinically for determining the presence of bile in the urine.

The Udranszki[6] *Viscosity Test for the Presence of Bile.*—Procure a sample of your urine and into each of two test-tubes place 5 mls. of the fluid. To A add 1 ml. of bile. Stopper each tube and shake them vigorously for one minute. Note the foam in A. The bile

[1] Unless there is some special agent to cause a regular arrangement. A current of electricity passing through the solution causes orientation.

[2] Page 115.

[3] The term "interface" means the plane separating two phases, that is, states of matter. Thus, the planes separating the air enclosed in soap-bubbles are interfaces.

[4] Surface tension has been put to practical use in synthesizing substances, like the antiseptic hexylresorcinol, $C_6H_3(OH)_2C_6H_{13}$, which, because of their power of lowering surface tension, are strongly penetrative and therefore germicidal. See Billard, G. 1906, Comptes rendus d. Sóc. de Biol., vol. 60, concerning surface tension and diffusion.

[5] M. Hay, Scotch physician of the 19th century.

[6] L. Udranszki, physiologist, Buda-Pest, Hungary, died, 1914.

contains mucus[1] which adds to the viscosity of the urine and permits the formation of foam. This exercise illustrates the Willard Gibbs principle:[2] To 5 mls. of urine containing bile add 1 drop of 1:1000 solution of furfural.[3] Stopper the tube and shake it as before. After a heavy foam has formed unstopper the tube and add 3 drops of concentrated H_2SO_4; the foam turns reddish, owing to a condensation of the aldehyde to form a colored compound.[4] The colored substance accumulates in the foam because it lowers the surface tension.[5]

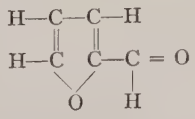
EXERCISE 8.—Select two small funnels of similar size and place each in an Erlenmeyer flask. Fit filter-paper into each funnel in the usual way. Fill each funnel with distilled water and drain as completely as possible. To one (funnel A) add 10 mls. bile; swing the funnel so that every portion of the paper comes into contact with the bile. Drain the bile from the funnel. Add 10 mls. of olive oil to each funnel. Note which funnel drains the faster. You will find that A does so, because the surface tension of the oil has been lowered by the bile.

Bile an Accessory to Enzyme Action.—Fat, taken into the stomach, undergoes little if any change unless bile has been regurgitated from the intestine, through the pylorus, into the stomach. The bile in this case lowers the surface tension of the fat and small globules of fat form from the larger masses, according to the Law of Plateau,[6] which predicts that spheres like oil drops tend to form such a shape that their surfaces (stress) are reduced to a minimum; the form which, for a given volume, presents the least surface is the sphere. The immense increase in surface of the fat globules, by emulsification, or division to form smaller spheres, permits the gastric lipase,

[1] Page 685.

[2] Page 115.

[3] The formula is:



[4] This phenomenon will be encountered throughout the following pages. The manufacture of bakelite and similar substances, used for fountain-pens, etc., employs the same principle of condensing aldehydes.

[5] The possibility of collecting substances that occur in small amounts in fluids by this principle has been investigated by commercial concerns abstracting enzymes from solutions; the enzyme in some cases has greater concentration in the foam than in the solution, so that it may be skimmed off with the foam.

[6] Belgian physicist.

or fat-digesting enzyme, to come into intimate contact with the oil. Bile, therefore, aids the fat-digesting enzymes in their work.[1]

EXERCISE 9.—*The "Peroxidase" of the Blood.*—The red coloring-matter of the blood contains iron, which produces nascent oxygen from peroxides:

$$H{-}O{-}O{-}H \xrightarrow{Fe} H{-}O{-}H + \tfrac{1}{2}O_2$$

Compare this equation with that on page 100 for the catalysis of H_2O_2 by platinum. The difference between these two reactions is that in the organic peroxides there is a receptor for the nascent oxygen, while in other cases two half-molecules of oxygen unite to form one oxygen. The following experiment illustrates this action; it is used in the detection of blood in urine, stools, on cloth, etc.:

Principle.—The acceptor of nascent oxygen is guaiaconic acid obtained from gum guaiacum. In the presence of oxygen this acid becomes guaiacum-blue.[2] Benzidine[3] and other substances may be used as oxygen receptor.

Procedure.—Transfer by means of a knife-blade tip a small amount of crystalline benzidine hydrochlorid to a test-tube. Add about 3 mls. glacial[4] acetic acid and mix. After the crystals have dissolved, add one volume of Merck's Superoxyl[5] or 5 volumes of the ordinary hydrogen peroxid 3 per cent.[6] Transfer one-half of the contents to another test-tube. To tube A add one drop of blood. Mix, and note the appearance of the blue color of the nitro-compound formed from the benzidine.

Other Consequences of Membranes on Particles.—Osmosis.—If a solution, confined by a semipermeable membrane (one which allows free passage to the solvent, but not to the solute) is placed in a solution of different concentration, a series of phenomena take place.

The solvent tends to pass from the less to the more concentrated solution. This passage is called *osmosis*.[7] If we prevent the change

[1] Another factor probably plays a part; the enzyme must dissolve in the fat if the fat is to be digested by the enzyme, and it is probable that bile-salts aid in this process.

[2] The reaction is given on page 397.

[3] Ibid.

[4] Crystallizable acetic acid, 99.5 per cent. pure.

[5] Hydrogen peroxid, 30 per cent. It must be handled with care.

[6] Hydrogen peroxid should be purchased in small containers and opened only as needed.

[7] Gr. *otheo*, push, *osmos*, a pushing.

in volume in the more concentrated solution by applying pressure, we find that for all non-electrolytes the amount of pressure needed is independent of the nature of the solute but varies directly with the concentration. The pressure necessary to overcome the tendency to an increase in volume is called *osmotic pressure.*

The first observations of osmosis were made on plant cells in salt solutions. When the cells were placed in such a solution that no change in cell volume occurred, the solution was said to be *isotonic*[1] with that of the cell. If the solution were of greater concentration it was called *hypertonic*[1] the cell would lose liquid and become shrunken. If the concentration were less, it was called *hypotonic*[1] and the cells imbibe liquid to such an extent that they finally become ruptured. In Exercise 11, these principles are illustrated by the use of blood cells. In 1877 the German botanist Pfeffer obtained quantitative measurements of these pressures by the use of artificial membranes. He caused a membrane of copper ferrocyanid to be deposited within the pores of a vessel connected with a mercury manometer. Ten years later van't Hoff, a Dutch physical chemist on the basis of Pfeffer's data enunciated the principle that his osmotic pressures were of the same numerical magnitude as those which would be obtained if the solute existed as a gas in the same volume and that therefore the laws of ideal gases held for osmotic pressures (π):

$$\pi = \frac{RT}{V_m} = \frac{n}{V}RT = cRT$$

in which RT is the work performed per mol at temperature T, V the molecular volume, n the number of molecules and c the concentration. From this expression, solutions which contain the same number of discrete particles in the same volume and at the same temperature have the same osmotic pressure. One gram-molecule of any gas at 0° C. and one atmospheric pressure occupies 22.4 liters. If the gas is compressed to a volume of one liter, it will exert a pressure of 22.4 atmospheres. In an analogous manner, one gram-molecule of any non-electrolyte contained in one liter of solution has an osmotic pressure of 22.4 atmospheres. Various other properties of such solutions will have similar values, thus the freezing point will be lowered 1.86° C (Δ = 1.86° C.); the boiling point will be raised 0.52° C. and the vapor pressure will be lowered 0.31 mm. mercury (20° C.). The above statements hold only for ideal solutions. The deviation is greater the more concentrated the solution. The error

[1] Gr. *isos*, equal; hyper, Gr. *'uper*, over; *'upo*, under.

arising is exactly the same for the various properties so that we can calculate the other properties if one is given. Thus,

$$\pi = \Delta\frac{22.4}{1.86}; \pi = 12\Delta \text{ (approx.)}.$$

In the above discussion, the solutions have been purposely restricted to non-electrolytes as it was shown by van't Hoff that strong acids, bases and salts always gave greater pressures than were expected. These compounds show identical deviations in all molecular properties of their aqueous solutions. van't Hoff proposed to bring these values into agreement with those calculated by inserting the factor i (the van't Hoff factor) into the equations:

$$\Delta_o = i\Delta_m c.$$

In a decimolar solution of NaCl, the observed Δ is 0.348° C. Substituting,

$$0.348 = i \times 1.86 \times 0.1$$
$$i = \frac{0.348}{.186} = 1.87.$$

As the dilution increases, this factor approaches 2.0 as a limit. In the case of $BaCl_2$, the limit approaches 3.0.

In 1887, Arrhenius proposed the theory that all solutions which were good electrolytes are dissociated to the extent that van't Hoff's factor indicates. A solution which gives rise to two ions like NaCl (Na^+ and Cl^-) is 100 per cent. dissociated in very dilute solutions and thus Δ is twice as great as if there were no dissociation. In a decimolar solution, the dissociation is equal to $i - 1$, or $1.87 - 1 = 87$ per cent. dissociated. For a decimolar $BaCl_2$ solution, the observed $\Delta = 0.48°$ C. In this case $i = 2.58$, that is, the solution acts as if it had a concentration 2.58 times that which would occur if there were no dissociation. As this increase is due to the formation of three particles in place of each molecule of $BaCl_2$, the excess above unity must be divided by 2 in order to obtain the degree of dissociation, $\frac{2.58 - 1}{3 - 1} = 0.79 = 79$ per cent. For a salt giving n ions, the degree of dissociation, a, is equal to $a = \frac{i - 1}{n - 1}$ At this point it must be said that while this theory as proposed by Arrhenius may be considered to hold for weak electrolytes it leads to marked discrepancies when applied to strong ones. It has been proposed by Debye and Hückel[1] to consider that all strong electrolytes are completely dissociated in solution and that variations in conductivity, freezing point depression, etc., are the result of changes in the mobility

[1] Debye, P. and Hückel, E. (see page 45). Physikal. Zeitschr., vol. 24, pages 185 and 305, 1923.

of the ions due to interionic forces. A new concept, called *activity* has been proposed by various workers to cover the deviations from the ideal state which occur in aqueous solutions, especially those of strong electrolytes. The activity of a solution may be considered that fictitious concentration which would have to be employed for the substance to have the same escaping tendency as an ideal gas.

Exercise 10.—Into a 100-ml. cylinder, filled with distilled water, add one crystal of potassium permanganate, $KMnO_4$. Let the cylinder stand without disturbing the contents. The dark permanganate begins to penetrate throughout the water; the process is known as diffusion. The diffusion pressure is that generated by the molecular vibration of $KMnO_4$ molecules. Being free to move throughout the liquid, they tend to distribute themselves throughout

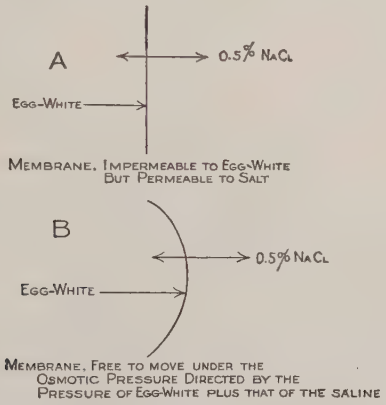

A
0.5% NaCl
Egg-White
Membrane. Impermeable to Egg-White But Permeable to Salt
B
0.5% NaCl
Egg-White
Membrane. Free to move under the Osmotic Pressure Directed by the Pressure of Egg-White plus that of the saline

Fig. 65.—Diagrams A and B show the development of osmotic pressure. Salt passes freely through the membrane, but egg-white cannot; there is, therefore, a pressure difference, with the result that the membrane is pushed outward.

the water, and equilibrium is reached only when the concentration of molecules and ions in one part of the cylinder is the same as it is in any other part. Note that diffusion pressure is directed centrifugally (Fig. 67).

Exercise 11.—*Osmotic Pressure Illustrated by the Fragility Test of Erythrocytes.*—Place in your test-tube rack 20 blood tubes,[1] 10 in the front row and 10 in the rear row.[2] Beginning with the lower left-hand tube, on the front row, label the tubes from left to right 0.6, 0.7, 0.8, 0.9, etc. Add to each tube the[3] number of mls. of 1

[1] Wassermann tubes, or small tubes holding about 5 mls.

[2] These tubes are for purposes of control and should contain a suspension of blood-corpuscles in isotonic (0.9 per cent.) NaCl solution. Ordinarily, this control is unnecessary and may be omitted.

[3] Using the 1-ml. Mohr pipette graduated in tenths of ml.

per cent. sodium chlorid solution[1] indicated on the tube. Drop into all the tubes the number of mls. of distilled water necessary to bring the total volume up to 2 mls.; that is:

Tube	1	2	3	4	5
Mls. 1 per cent. NaCl	0.6	0.7	0.8	0.9	1.0
Mls. dist. H_2O	1.4	1.3	1.2	1.1	1.0
Percentage salt concentration	0.30	0.35	0.40	0.45	0.50

Tube	6	7	8	9	10
Mls. 1 per cent. NaCl	1.1	1.2	1.3	1.4	1.5
Mls. dist. H_2O	0.9	0.8	0.7	0.6	0.5
Percentage salt concentration	0.55	0.60	0.65	0.70	0.75

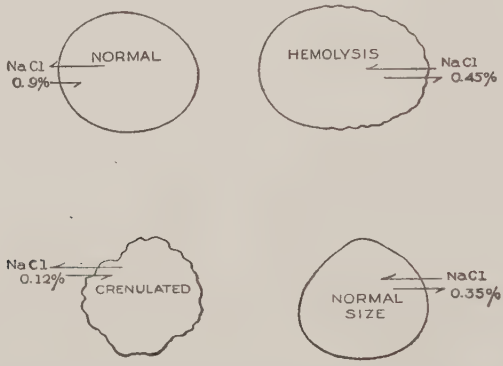


Fig. 66.—Upper left: Isotonic relations; H_2O passes freely in either direction through cell membrane. Upper right: Hypotonic relations; lower concentration without than within; consequently, accumulation of liquid within and bursting of membrane. Lower left: Hypertonic conditions; greater concentration without and hence withdrawal of liquid to outside, with shrinkage of cell membrane. Lower right: Corpuscle able to resist changes in tonicity relations; although there is a concentration of 0.35 per cent. NaCl without, the membrane does not rupture. This is the basis of the "fragility test" for corpuscles (page 121). Compare also Fig. 65.

The arrows represent the passage of water and diffusible substances. The length of the arrow indicates the relative rate of diffusion. (The sodion does not enter the corpuscle.)

Now, to each tube in the front row, add 0.1 ml. blood. Mix by rotating the tube between the palms of the hands. Note in which tube the blood has hemolyzed and in those tubes in which it has not; this is indicated by the clearness of the red liquid in hemolyzed and the murkiness in tubes in which the blood has not been hemolyzed. Normal blood usually hemolyzes at 0.45 per cent. NaCl. The test

[1] Weigh 10 grams of highest purity NaCl crystals and dilute to 1000 mls. with distilled water.

is used in clinical diagnosis for the purpose of distinguishing different forms of jaundice.[1]

The Factors of Osmosis.—The osmotic pressure in the saline solution in which the erythrocytes are suspended becomes too high or too low; the pressure in the corpuscle becomes decreased or increased. Rupture of the stroma of the corpuscle results. Thus the hemoglobin is freed to the surrounding plasma or saline solution. If the osmotic pressure within and without the corpuscle is the same, then the condition is isosmotic. If the pressure within is greater than without, then the outer liquid is hypotonic with respect to that within the corpuscle. If the pressure within the corpuscle is less than that without, then the solution without the corpuscle is hypertonic. Ions

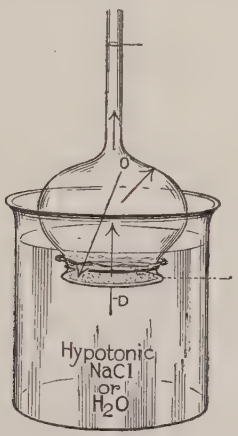


Fig. 67.—An osmometer. Water or other hypotonic solution is placed in the beaker and a fluid, one or more components of which cannot pass through the membrane, in the thistle-tube. The arrows within the tube show the direction of osmotic pressure the only release for which is the passage of the liquid up the narrow tube. Hence the rise of liquid in the osmometer.

pass through the membrane in both directions until equilibrium is reached. If there are fewer molecules outside the corpuscle than within, water, bearing the ions and molecules, flows until the hydrostatic pressures are the same on both sides of the corpuscular membrane. A practical illustration of this phenomenon is seen in the use

[1] If blood of icterus patients is available, the test may be made interesting; add 0.1 ml. patient's blood to each tube in the rear row, mix, and compare with that in the front row (control). Jaundice, in which there is no bile in the urine, exhibits a fragility greater than normal (above 0.45 per cent.). In obstructive jaundice of the prevalent catarrhal form fragility is lessened and hemolysis does not occur in saline stronger than 0.45 per cent.

of saline purgatives[1] (Fig. 68). A salt of low absorbability like magnesium sulphate (Epsom salt) is given by mouth. It passes into the intestine (A) as a mass of molecules and ions ($MgSO_4$; Mg^{++}; $SO_4^{=}$). The mass is hypertonic to the molecular and ionic concentration in the blood, and since $MgSO_4$ cannot pass very quickly through the membranes, water must flow into the intestine from the blood until the hydrostatic conditions are in equilibrium (B). This is indicated in the diagram (Fig. 68) by the equal spacing of the dots in the blood and in the intestine. Water, thus introduced from the blood, aids in the movements of the feces.

The factor of inequilibrium of ions, due to the retention of some of these bodies by the substance of the corpuscle, according to the theory of equilibria of Donnan,[2] comes into play here also. Molecules

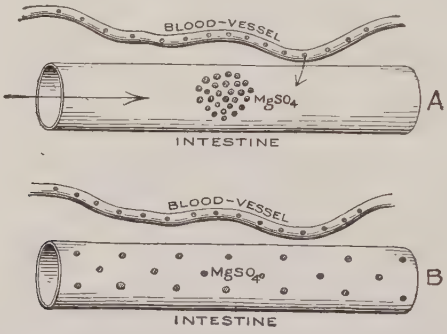


Fig. 68.—Diagrams to show the physical chemistry of saline purgatives. Magnesium sulphate is administered in large doses and reaches the intestine; the sulphate is but slowly absorbed into the tissues; owing to the differences in concentration water flows from the blood into the intestine until equilibrium is established. The redistribution of the particles after equilibrium has been reached is shown in the lower diagram. $MgSO_4$ above is hypertonic; compare Fig. 67.

and ions accumulate on the inside of the corpuscle, chemically bound to the contents of the corpuscle.

Methods for the Determination of Surface Tension.—Two chief methods for measurements of surface tension are in use, namely: (1) the drop-weight method and (2) the direct method. The first method employs the stalagmometer; the second, some form of delicate balance which measures the force necessary to cause a disk to be torn from the surface of the liquid under discussion. Quantitative determination of surface tension:

[1] The osmotic process is not the sole cause of the purgative action of saline cathartics. See Sollmann, T., Pharmacology, Philadelphia, W. B. Saunders Co., 1923.

[2] Page 104.

(1) **By Means of the Stalagmometer.**[1]—*Principle.*—The size of a drop of liquid falling from a pipette depends upon the surface tension which counteracts gravity. Figure 69 shows the conventional stalagmometer. For the purposes of this book the following Exercise may serve:

EXERCISE 12.—Count the drops of freshly distilled water at 20° C. falling from the end of an Ostwald-Folin 1-ml. pipette[2] until the full 1 ml. of water has passed. Dry the pipette with ethanol 95 per cent., followed by ether and the air-blast. Repeat, filling the pipette with olive oil. Calculate the surface tension from the following formula, expressing the result in ergs[3] of energy:

$$\frac{\text{Number of drops of water} \times \text{specific gravity of oil}}{\text{Number of drops of oil}} \times \text{gravity constant.}$$

The gravity constants for different locations are given in the Appendix; specific gravity, also in the Appendix.

The principle may be illustrated by the following Exercise (Worsnop, B. L., and Flint, H. T., Advanced Practical Physics, New York, E. P. Dutton & Co., 1925, p. 125):

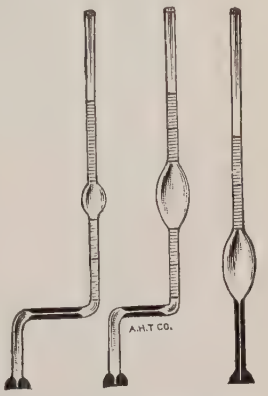


Fig. 69.—Stalagmometer for determining surface tension by the drop-volume method. Left to right, tube for thin liquids; larger quantities of thin liquids, and, to the right, tube for viscid liquids like blood.

(2) **By Means of the Torsion Balance** (Fig. 71).[4]—*Principle.*

EXERCISE 13.—Place the small specific gravity bench (Fig. 70) over the left pan of the balance, being careful that it does not touch it. Fasten to the hook from the end of the beam a thread bearing a piece of nichrome-wire or other platinum substitute. The wire is bent into a wide, shallow U and fastened to the thread by its middle, so that, when dipped beneath the surface of the solution under examination, and then withdrawn, it will carry with it a

[1] From the Greek *stagon*, to drop. Stalactites are accumulations of precipitated limestone in caves. They are attached to the roof and gradually increase in size as the water, bearing dissolved limestone, passes over them and, by evaporation, the limestone is deposited.

[2] The pipette must be cleaned as described on page 189.

[3] The unit of work. An erg is the work done when 1 gram is moved through the distance of 1 cm. with 1 dyne of force.

[4] For the application of the use of this principle, to practical medicine, see Leonard, V. and Wood, A. (Johns Hopkins University, Baltimore, Md.) J. A. M. A., vol. 85, p. 1855, 1925.

film of liquid between the prongs of the U.[1] Then determine by weighing how much mass is added to the wire by the adhering liquid when weights are added to the right pan until the beam is horizontal. Then the surface tension in dynes, T, equals the added mass m (obtained by counting the counterpoised weights in the right pan) times the gravity constant g, for the place,[2] divided by twice the length of the wire between the prongs, l, measured in centimeters; that is:

$$T = \frac{m \times g}{2 \times l} \text{ dynes per centimeter.}$$

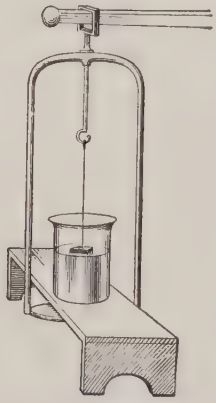


Fig. 70.—Showing arrangement for roughly determining surface-tension by means of a laboratory balance. By applying weights to the opposite pan of the balance, until the disk breaks away from the surface of the liquid, the surface-tension of which is sought, the dynes of force exerted by the surface may be estimated. This is the principle of the du Noüy method (Fig. 71).

The *du Noüy torsion balance*[3] for surface tension determinations (Fig. 71[4]). A platinum ring (Fig. 71, *R* and Fig. 72) is attached to a lever (*K*) which is fastened to the torsion wire after it passes across the rest (*I*). The torsion wire is controlled by the milled head (*D*) and the extent of torsion is indicated on the dial (*S*). The platinum ring (*R*) is brought into contact with the liquid under examination in the dish placed on the platform (*T*). The dial (*S*) registers the amount of torsion necessary to cause the ring to break from the surface of the liquid. This amount of torsion is then converted into weight by

[1] The thread is attached to the rounded part of the U and hence the wire is suspended like the figure ∩.

[2] Appendix.

[3] While the description is written for the du Noüy apparatus, the Searle (Pye & Co., Cambridge, England) apparatus, designed earlier, can be understood at the same time.

[4] The author is indebted to the Central Scientific Co., Chicago, who make the instrument, for the use of the figure.

weighing a circle of filter-paper which just fits into the ring, and when the disk is in place on the ring, by adding weights to it until the indicator on the dial (*S*) registers just zero. The combined weight of

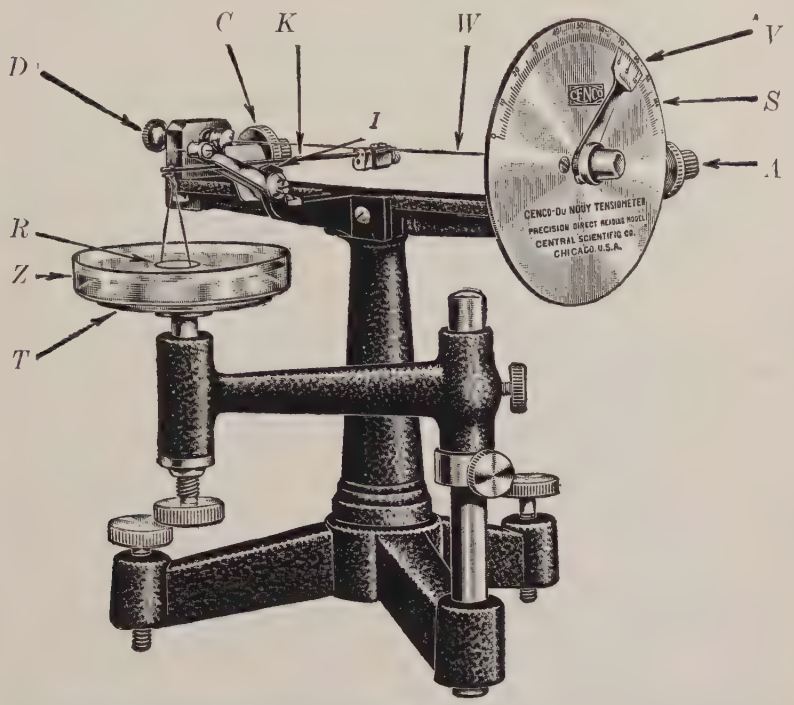


Fig. 71.—Apparatus for determining surface tension. The tension exerted by the surface of the liquid in the shallow dish, *Z*, upon the platinum ring, *R*, when tension is applied by the torsion of the wire, *W*, operated by the arm, *V*, is estimated in work units on the calibrated disk, *S*.

Fig. 72.—The disk used in the direct method of determination of surface tension (see the du Noüy method, Fig. 71) to show the tension of the surface film within and that without the ring, a necessary consideration in calculations.

the paper and of the metal weights is the weight necessary to break through the surface. The unit of surface tension is the same as

before, the dyne. Gravity exerts a force of 980.15 dynes in Philadelphia. The ring is 4 cms. in circumference, that is, 4 cms. of platinum come into contact with the liquid. Moreover (Fig. 72), the pull of the surface is exerted twice, once upon the wire from within the ring, and again upon the wire from outside the ring. The dynes of force exerted upon the surface by its tension are:

$$\frac{\text{Combined weight of paper and weights in grams} \times \text{gravity constant}}{2 \times 4}$$

Precautions.—The tension wire must be kept clean (cleaning solution, Appendix), likewise the watch-glass must be free from grease. Determination of the zero: Bring the indicator (*V*) to zero. Turn the milled head (*A*) until the lever is just free from the rest (*I*). Tighten the screw (*C*). Loading the apparatus: Place the watch-glass upon the platform (*T*), and by means of the adjusting milled head below the platform bring the surface of the liquid in the watch-glass so that it just touches the ring (*R*). Making the determination: Turn the milled head (*A*) carefully until the ring becomes free from the surface of the liquid. Read the dial (*S*). Calibrate the dial: By means of a large cork-borer cut disks of filter-paper to fit the ring (*R*). Weigh one disk accurately. Place the disk upon the ring and balance with weights until the indicator (*V*) comes back to zero. The calculation is given above. The surface tension of normal urine is about 50 dynes per centimeter; that of distilled water about 77 dynes per centimeter.

The Behavior of Colloids toward Water.—Colloidal substances may be divided into two classes according to their relation to water:

Hydrophobic[1] colloidal solutions:	Hydrophilic[2] colloids:
The substance does not absorb water. Ex.: Au, Pt-suspensoids.	The substance absorbs water. Ex.: Egg-white; gelatin.

Hydrophilic colloidal solutions are used to peptize hydrophobic colloidal solutions, an example of which is the use of egg-white in the making of French dressing (olive oil and vinegar); egg-white protects the colloidal oil-drops from coalescing. It is possible to precipitate a hydrophilous colloidal suspension, like egg-white, by introducing a dehydrating agent. The following Exercise is designed to show this process:

Exercise 14.—Place 10 mls. of 1 per cent. egg-white in a test-tube and add crystals of ammonium sulphate $(NH_4)_2SO_4$ to saturation.[3] Filter. Test the filtrate for egg-white by adding one drop of

[1] From the Greek *hydor*, water, and *phobos*, fear.

[2] From the Greek *hydor*, water, and *philia*, affection.

[3] Eight grams ammonium sulphate crystals at room temperature (20° C.).

1: 10 acetic acid solution and boiling; there should be no appearance of cloudiness; if such appear,[1] repeat, using about 2 gs. more of the sulphate for the whole of the filtrate.

Ammonium sulphate behaves here as does magnesium sulphate when used as a saline purgative (page 124); it absorbs water. Egg-white exists as a colloidal solution partly because of hydrostatic properties, and if equilibrium between the amount of water in the egg-white and that in the surrounding medium is disturbed, as in the experiment described above, precipitation occurs. Such processes are called "salting-out." The principle will be utilized later (page 223) to separate some of the meat-like substances from each other.

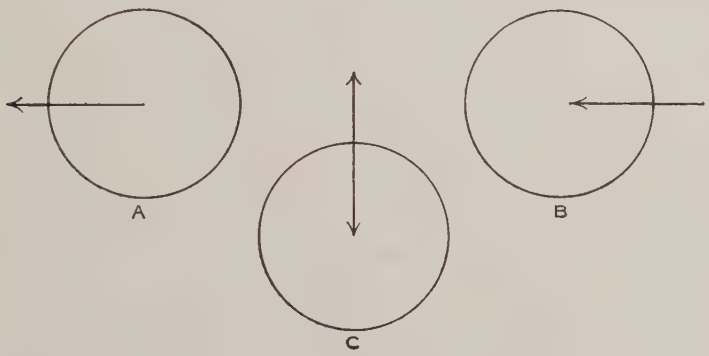


Fig. 73.—A, Condition of hypertonicity; B, hypotonicity; C, isotonicity. Compare Fig. 66.

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(*b*) See Bogue, vol. 1, p. 23.

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[1] Owing, as a rule, to incomplete saturation.

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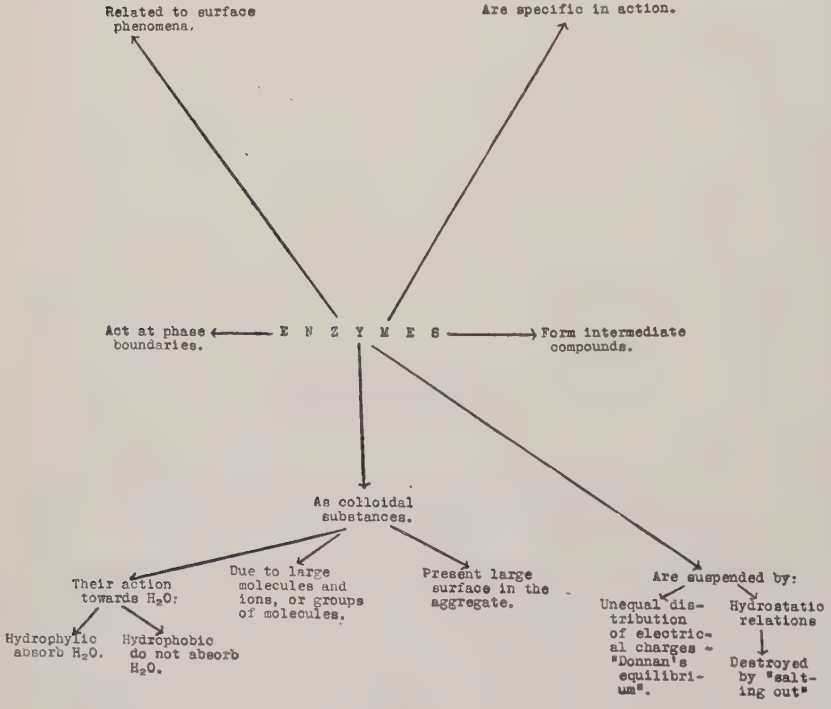
GRAPHIC SUMMARY FOR ENZYMES.


Related to surface phenomena.
Are specific in action.
Act at phase boundaries.
E N Z Y M E S
Form intermediate compounds.
As colloidal substances.
Their action towards H_2O:
Hydrophylic absorb H_2O.
Hydrophobic do not absorb H_2O.
Due to large molecules and ions, or groups of molecules.
Present large surface in the aggregate.
Are suspended by:
Unequal distribution of electrical charges = "Donnan's equilibrium".
Hydrostatic relations
Destroyed by "salting out"

CHAPTER III

THE BODY AND ITS MAINTENANCE

"Like studying life through creatures you dissect—
You lose it the moment you detect."

Pope, "Essay on Man."

Body Structure.—The human body is essentially like all organisms in its chemical structure, for by the great *Law of Similar Function*, all organisms perform certain fundamental functions, which are either for the maintenance of the individual or of the race; that is, nutrition or reproduction. There are functions accessory to the former: locomotion, employed in searching for food; sensation, which correlates the different parts of the body; digestion, by which process foods, which are typically colloidal, become crystalloidal and are made to pass through membranes into the blood; excretion, or the elimination of substances which become more or less completely oxidized, or serve to remove such compounds. For the purposes of reproduction specialized tissues have as their function the production of individuals similar to those whence they came. Affiliated with reproduction is growth, by which the individual reaches the proportions of the parent.

Specialization.—A specialization of parts of the body exists in order that various reactions may be performed in a regular manner. This morphological specialization is accompanied by a chemical differentiation, which is to be expected, since chemical behavior determines both functional and morphological states. Certain qualities accompany chemical configurations: no matter where we find the radicle OH we find a characteristic behavior. It is not always possible to tell what function a chemical substance or radicle plays, because we do not have enough knowledge of the substance to predict what it may do. For instance, creatin[1] always occurs in muscle, but we do not know its precursors, nor what relation it has to muscle action. Nevertheless, we believe that it is concerned in some way with this action, owing to its invariable presence in muscle. Specialization of

[1] Page 364. Fiske has shown that creatin exists in muscle in combination with phosphoric acid, on an ester of the same.

chemical constitution is again illustrated by the fats of adipose tissue, and by those of the blood and nervous system. Neutral fats are

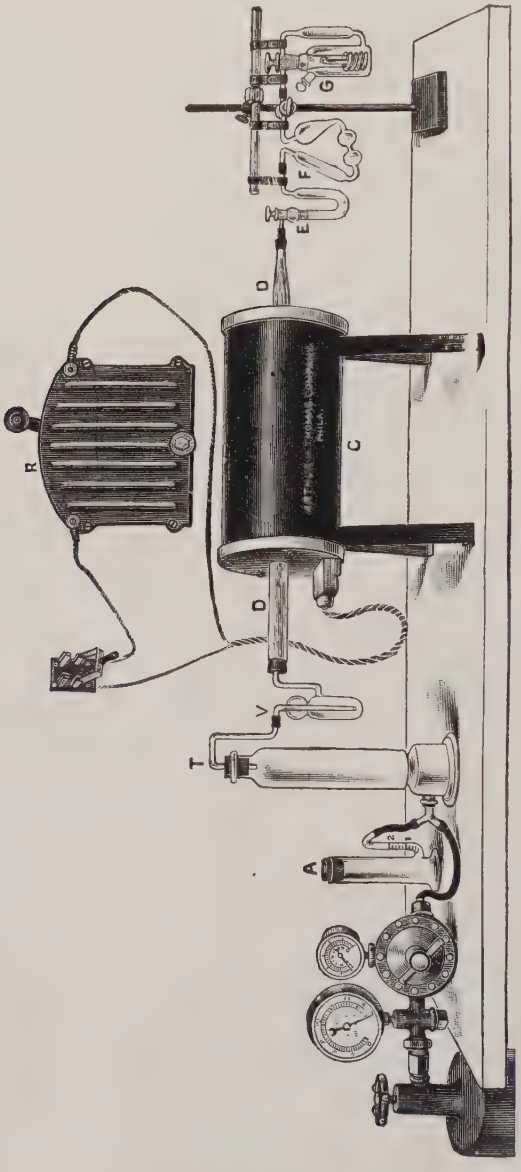
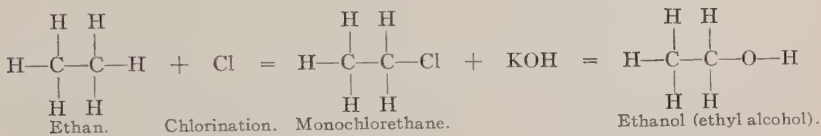


Fig. 74.—Apparatus for determining the elementary composition of organic substances of biochemical importance. Left to right: Oxygen tank; gas controllers; wash-bottles; electric furnace carrying the combustion tube (*D*); absorbers for CO_2 and H_2O. The wall-rheostat controls the current heating the furnace.

characteristic of adipose tissue; whereas only phosphorized fats occur in the brain and in the blood, the fats are largely phosphorized.

The Foods.—The body cannot utilize as food, substances which have great chemical activity. The teeth contain fluorin, the most active of the elements. In the body its activity is limited by being combined with calcium to form calcium fluorid. For the same reason foods enter the body in partly oxidized form. The glucids[1] are hydroxy-compounds; likewise the fats. The protids[2] are similarly hydroxy-compounds, with ammonia. All materials used by the body as food resemble it in chemical composition, and comparatively slight variations from this composition render substances undesirable as food. For instance, the hydrocarbons are not used as food; yet the addition of a single atom of oxygen to ethane, making ethanol, converts the hydrocarbon to a food substance.[3]



Sources of Foods.—The human body cannot derive its energy from inorganic foods.

It must depend upon organisms which can utilize inorganic substances, such as the green plants, which contain chlorophyl,[4] chemically similar to hemoglobin the coloring-matter of the blood. Chlorophyl sieves the light, permitting only rays of definite wave length to enter the cells. Chlorophyl absorbs light in the region of the red rays[5] and also of the violet rays.[6] At these points, where light is absorbed to the greatest extent, the optimal chemical activity occurs, according to the Draper-Grottus Law,[7] which predicts that light must be absorbed to be effective in chemical reactions, as well as in electrical and other types of reactions.

Synthesis of Foods from Inorganic Sources.—Photosynthesis[8] is seen in the conjugation of carbon dioxid and water to form form-

[1] Carbohydrates; they are the starches, sugars, substances like paper, etc.

[2] Meat-like substances, muscle, beans, cheese, etc.

[3] Speaking in general terms; all alcohols are poisonous, especially methanol.

[4] Greek *chloros*, green, and *phyllon*, leaf, "leaf-green."

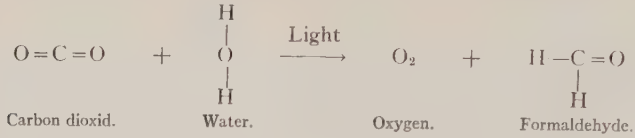
[5] 6500 Å (Ångstrom units). One unit is the wave length of light measured in ten millionths of a millimeter.

[6] 4250 Å.

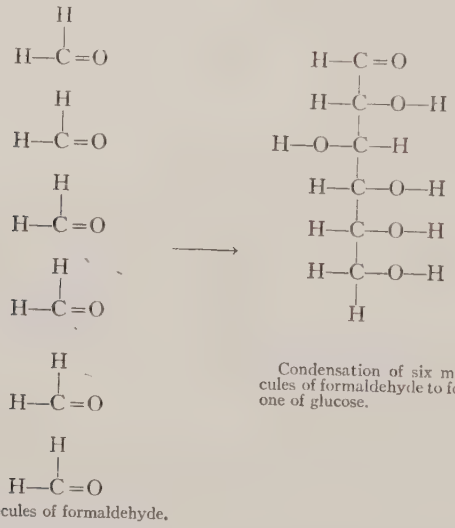
[7] J. W. Draper, pioneer chemist of the United States in the Free Academy, now College of the City of New York. Grottus, C. J. T., German chemist, 1819.

[8] Greek *phos*, light, and *syn*, together; that is, a synthesis by means of light.

aldehyde, which becomes condensed to form sugar. Oxygen is evolved. The general reaction may be given as follows:



Formaldehyde may then be condensed into glucose:



Six molecules of formaldehyde.

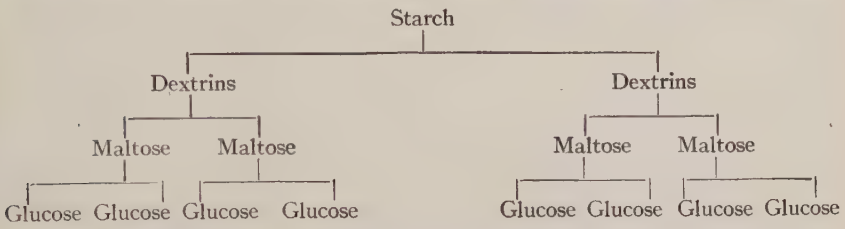
Condensation of six molecules of formaldehyde to form one of glucose.

The optimal wave length of photosynthesis is about 2200 Å. Waves shorter than 1900 Å destroy formaldehyde. This wave length has been determined experimentally by moving a quartz-mercury-vapor lamp toward and away from the vessel holding the CO_2 and water. Waves that are deleterious to formaldehyde are screened by colloidal iron. Formaldehyde is produced by spraying from an atomizer carbonized water, like that used in soda-fountains, before a quartz-mercury lamp.[1] If this vapor is caught and, in turn, exposed to the light, sugar is formed according to the structural formulæ already given. It is possible, therefore, experimentally to imitate photosynthesis in the green plant. In order to protect the sugar thus formed in the plant the glucose is dehydrated and further condensed to starch:

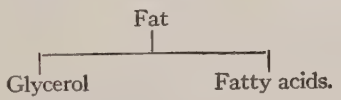
$$\underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Glucose.}}{C_6H_{12}O_6} \ . \ . \ . \rightarrow \underset{\text{Starch.}}{(C_6H_{10}O_5)_n} + \underset{\text{Molecules of water.}}{(H_2O)_n}$$

[1] The reason that quartz is used is that it is permeable to such waves as these. Ordinary glass is opaque to shorter wave-length light. The quartz-mercury lamp is used in therapy in rickets, etc. See page 559.

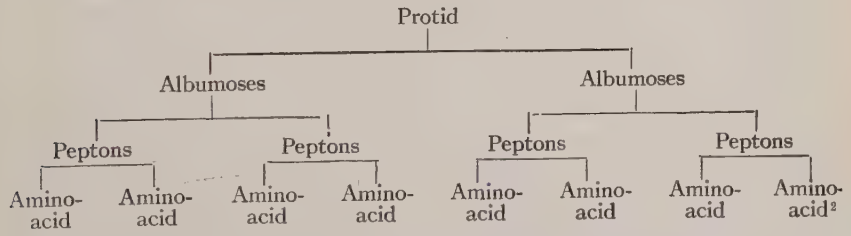
The Fundamental Chemical Structure of Foods.—Each energy-producing food substance is resolvable into typical structural units, or is already in unit form. During digestion the process of fragmentation of the compound sugar, fat, or protid occurs. Starches are digested to sugar (glucose). One may think of starch as a building composed of bricks, the foundation bricks being molecules of glucose:



Two molecules of glucose condense to form maltose; the maltose is in turn condensed to form the dextrins, and the dextrins to form starch. We designate the products of starch digestion, collectively, as *glucidtemns*.[1] Again, the fats are composed of building stones, the alcohol, glycerol, and fatty acids:



Glycerol and the fatty acids are collectively known as *lipidtemns*. Similarly, the protids or the meat-like, nitrogenous substances are digested to simple acids, the *amino-acids*, through a group of intermediate substances analogous to the dextrins and maltose of the glucid series:



The Mode of Union of the Fundamental Chemical Units into the Molecules of the Foods.—There is a simple plan throughout the chemistry of foods whereby the various constituents, glucidtemns, lipidtemns, and protidtemns, are condensed into the molecules of

[1] Greek *glucid*, and *temno*, to cut; that is, the dextrins, maltose and glucose, are the products "cut" from the glucid, starch, by chemical action during digestion.

[2] This diagram is purely arbitrary. Actually, in all protids, many amino-acids are found in each pepton.

starches, fats, and protids. The process is known as *dehydration synthesis;* a molecule of water is removed for each condensation:

$$\text{Glucose} + \text{glucose less } H_2O \longrightarrow \text{maltose.}$$

During digestion water is restored:

$$\text{Maltose} \xrightarrow{H_2O} \text{glucose} + \text{glucose.}$$

Similarly for the fats:

$$\text{Glycerol} + \text{fatty acids less } H_2O \longrightarrow \text{fat.}$$

During digestion water is replaced:

$$\text{Fat} \xrightarrow{H_2O} \text{Glycerol} + \text{fatty acid} + \text{fatty acid} + \text{fatty acid.}$$

For the protids:

$$\text{Amino-acids less water} \longrightarrow \text{protid.}$$

In the digestion of protids:

$$\text{Protid} \xrightarrow{H_2O} \text{amino-acid} + \text{amino-acid} + \ldots.$$

Assimilation.—The term "assimilation"[1] has been used for years to designate the process whereby the digested products become a part of the body. Our present knowledge permits us to use the term in a more critical sense. The products of the digestion of starch are absorbed into the blood and are distributed as glucose throughout the body. It is probable that in the active man much of the glucose is destroyed at once, but some is converted into animal starch[2] and stored in the liver and muscles. Other sugars, like milk-sugar, are converted into glucose, utilized at once, or excreted. Fats may be carried in the blood as neutral fats, or in special combinations with phosphoric acids or with cholesterol.[3] The "neutral" fat may be stored in the adipose tissues, or used at once. The amino-acids are sent through the blood-stream and are absorbed by the various tissues. The amino-acids which can replace those lost in the wear and tear of the muscles, etc., are incorporated into the substance of these tissues, and those which cannot be used are returned to the blood-stream and disposed of.

[1] Latin *ad*, to, and *similis*, like; that is, "like unto."

[2] Glycogen, see page 190.

[3] The substance of which types of gall-stones are made, see page 198.

The Uses of the Foods.—Foods have two principal uses: (1) *To provide heat* which is necessary to bring the body to the optimum temperature for enzyme action, and to compensate for the loss of heat by evaporation, etc. Heat is necessary to maintain the constant temperature of the normal body (37° C.; 98.6° F.). This temperature is the optimum for almost all enzymes occurring in the body,[1] and must be maintained for the proper functioning of the organism.

(2) *To repair the structures of the body* destroyed in its activities. Glucids, lipids, and one portion of the protid are used for the production of energy. Fats afford the greatest amount of heat.[2] The nitrogenous portion of the protids serves to restore the nitrogen that is lost in the urine.[3]

Excretion permits the body to free itself from the products of metabolism, and from substances which, not being useful, pass unchanged through the body. Excretion has three aspects:

(1) Excretion of substances like inorganic salts which have formed a part of the tissues, but, having played their part, are thrown into the blood-stream and conducted thence to the kidneys to be excreted in the urine. The reason for this is not known, save in certain cases.

(2) Excretion of the end-products of combustion in the body. These (CO_2; H_2O) are the "ash" of the fires.

(3) Excretion of substances to maintain the osmotic pressure or reaction equilibrium of the body. Sodium chlorid[4] is an example of the former group. Salt is introduced daily into the body in the foods and would accumulate if some means were not provided for its elimination. Acid phosphate, NaH_2PO_4, is excreted in order that the reaction of the blood be maintained at near neutrality (pH 7.3). By the daily elimination of a certain portion of these inorganic substances, their concentration, for osmotic purposes, is maintained at a fairly definite level.[5] While the urine is the principal means of void-

[1] And, strangely, for enzymes occurring in organisms that do not maintain a constant temperature, like the so-called "cold-blooded" animals, which should be termed variable-temperatured organisms, for they adopt the temperature of their surroundings (poikilothermous animals).

[2] In making this statement the author has not forgotten the discrepancies pointed out by Professor Graham Lusk in the modern conceptions of heat production in the organism and the explanation of muscle movement. (See page 145.)

[3] About 12 gms. of nitrogen are lost from the body through the urine each day, and protids must be fed to compensate for this loss.

[4] We have said above that the reason for the loss of inorganic substances is unknown. It is. We do not know save in the case of one or two why they are not saved.

[5] About 15 grams of NaCl are taken into the body daily and an equal portion is excreted through the urine, perspiration, etc. By these means the amount of NaCl in the blood is maintained at a constant level (0.5 g. per 100 mls. blood).

ing these substances, the lungs excrete CO_2 and H_2O, which are end-products of the metabolism of all three of the chief organic food stuffs. The lungs also aid in maintaining the neutrality of the body by eliminating CO_2 (carbonic acid, H_2CO_3). The feces consist largely of the remains of bacteria, the secretions (mucus, enzymes, etc.) of the alimentary tract, and certain substances like iron and calcium which have been a part of the body. The fat of the feces is not simply body fat, but is a true excretion product. The skin excretes water, some inorganic salts, and nitrogenous substances like urea. From the glands in the mouth uric acid is excreted to some extent. The kidney, however, is the chief excretory organ of the body.

The Accessory Substances of the Foods.—We have spoken of the inorganic salts which accompany the foods; these do not give rise to energy, but in various ways aid in its production. Certain organic substances or radicles, like the aromatic ring, , accompany the foods, but the power of the body to cleave the ring and thus render the substance available as food is small, and from the standpoint of nutrition, negligible. These organic substances are combined with sulphuric acid, or with an acid derived from glucose and excreted in this conjugated form, which is a harmless substance, while the ring involves dangerous possibilities. After metabolism has robbed the ring of its side-chains, a poisonous substance, phenol, , is usually left. Many cases similar to this will occur in biochemistry. Among the accessory foods may be included the vitamins, the chemical nature of which we are just beginning to learn. The minute amounts in which they seem to function does not warrant classifying them as energy producers, yet energy is produced in abnormally low amounts in their absence.

Inorganic Salts.—These compounds are utilized in the body to keep certain substances in solution; to aid in enzyme activities; to aid in certain phenomena, like the clotting of blood and the curdling of milk to which calcium is indispensable. Structures which behave rhythmically, like the heart and cilia, require a very definite concentration of inorganic substances in the medium in which they work. The development of certain structures, like the bones, is dependent upon the presence of properly balanced concentrations of inorganic salts. Rickets, a disease of the child and occasionally of the adult, bears a definite relation to the calcium and phosphorus content of

the blood. Sulphur occurs in cystin, which is one of the amino-acids resulting from the breaking down of meat-like substances. The function which sulphur performs in this way is unknown, but recently it has been shown that it probably has part in oxidation within the tissues. Iron occurs in the blood, where it undoubtedly serves as a peroxidase.[1] Iodin, found in minute amounts throughout the body, occurs in largest amounts in the thyroid gland; the function of iodin is not clear, but this element is contained in the molecule of a special substance, thyroxin, which plays a most important rôle in the organism both in health and in disease.

Inorganic Constituents of the Body.—The following inorganic substances as elements have been found in the human body; note that the statements made on page 96 concerning the elementary composition of the body are illustrated below.

Atomic number.	Element.	Characteristic distribution.	Atomic weight.
1.....	Hydrogen	Throughout, as H_2O.	1.008
3.....	Lithium	Lungs, etc.	7.010
6.....	Carbon	Throughout.	12.005
7.....	Nitrogen	Throughout in protid.	14.008
8.....	Oxygen	Throughout as H_2O.	16.000
9.....	Fluorin	0.2 per cent. bone-ash.	19.000
11.....	Sodium	Throughout as NaCl.	23.000
12.....	Magnesium	Throughout less than Ca in lungs, kidney, liver, and glands; greater than Ca in brain, muscle, and heart.	24.320
14.....	Silicon	Lens, 0.05 per cent.; muscle, 0.002 per cent.; skin, 0.004 per cent.	28.000
15.....	Phosphorus	Phospholipids; phosphoprotids; in urine, acidity.	31.04
16.....	Sulphur	In cystin (hair), 0.5 per cent.; liver, 0.9 per cent.	32.060
17.....	Chlorin	Throughout as NaCl; in cytoplasm rather than in nucleus. Blood, 0.2 per cent.; kidney, 0.1 per cent.; bile, 0.01 per cent.	35.460
19.....	Potassium	In cytoplasm. Blood-serum, 0.2 per cent. as K_2O. About 20 per cent. less K than Na at birth in the total body. Muscle has six times as much K as Na. Liver, two times as much.	39.100
20.....	Calcium	Typically in nuclei of cells. As CaO, muscle has 0.005 per cent.; lung, 0.001 per cent.; kidney, 0.008 per cent.	40.070
25.....	Manganese	Accompanies iron in many tissues. As MnO_2 blood contains 0.00005 per cent. In ash of pancreas, 2.2 per cent.	54.930
26.....	Iron	Throughout in amounts under 0.01 per cent. Human fetus, 0.14 mg. per cent.; liver, 0.1 mg. per cent.; more in female.	55.84
27.....	Cobalt	Blood (Macheboeuf).	58.94

[1] Page 118.

Atomic number.	Element.	Characteristic distribution.	Atomic weight.
28.....	Nickel	Blood (Macheboeuf).[1]	58.69
29.....	Copper	Kidney, 0.001 per cent.; liver, 0.0002 per cent. Traces in blood.	63.57
30.....	Zinc	Liver, 0.001 per cent. to 0.007 per cent.	65.37
33.....	Arsenic	Thyroid, 0.000,007 per cent.; brain, 0.000,001 per cent.; liver, 0.000,001 9 per cent.	74.96
53.....	Iodin	Thyroid, 0.04 per cent.; liver, 0.001 per cent.; brain, 0.002 per cent.	126.92

It is seen that no inert element, like argon,[2] occurs in the body; that radio-active elements and those that are undergoing decompo-

Fig. 75.—H. C. Bradley, Professor of Physiological Chemistry, University of Wisconsin, Madison. Investigations concerning the rôle of enzymes in biochemical processes, especially those of tissue ("autolytic") enzymes, the function of lipases in fat metabolism; earlier investigations concerning the presence of inorganic substances like manganese in organisms.

sition are lacking; and that with regard to atomic weight, iodin is the farthest up the scale. Heavy elements, such as lead, and the noble metals, are not found. Two explanations may be offered:

(1) The distribution of the elements in the organism is an historical matter, representing the period in evolution when only those

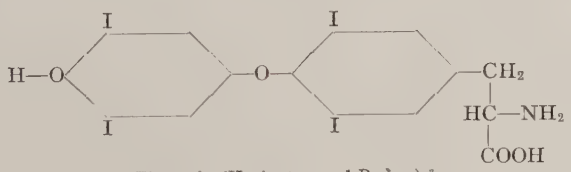
[1] Bertrand (p. 349) found a high content of cobalt and of nickel in the pancreas and in insulin in a concentrated form. For the mechanism of the process involved see page 511.

[2] Argon accompanies the air into the lungs as nitrogen does, but in both cases they play no part in the economy of the body.

elements that are of lighter weight than iodin were evolved. This is not probable.

(2) The lighter kinds occur in living things because these elements were relegated to the surface of the earth and were available for the use of the organism as it has undergone evolution. The geologist believes that the heavier elements lie toward the center of the earth, since the total weight of the earth demands heavier substances near the center of mass.

Correlative Systems of the Body.—In an organism as complex as the human body special means are required to adjust the various parts to each other. Two great systems exist for this purpose: the *nervous* and the *chemical*. The first is practically a telegraph system. The second is known as the endocrine system, or the system of internal secretions.[1] This subject will be considered in more detail later in the present volume, but one or two examples may be given here. Iodin has been mentioned as a constituent of the thyroid gland. The substance which is present in the gland and which is doubtless its characteristic ingredient is thyroxin.[2] This substance has the



Thyroxin (Harington and Barger).[3]

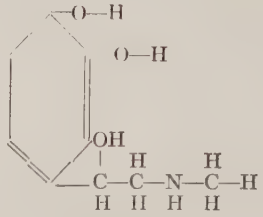
power of accelerating metabolism, according to the mechanism described on page 99. If 1 mg. for every 1000 gms. of body weight be introduced into a normal person, a 2.8 per cent. increase in utilization of oxygen results. This indicates that the function of the substance is to maintain the oxidation of the body, regardless of whatever may interfere with the normal impulse to burn the foods. When too much thyroxin is formed the body is on a heightened plane of metabolism and serious pathological changes, known as hyperthyroidism, a type of goiter, develop. On the other hand, thyroxin may not be produced in sufficient quantities to maintain the proper oxidative impulse, and hypothyroidism, occurs.

[1] Page 635. It is also called the cryptorhetic and the hormone system.

[2] See Jour. Amer. Med. Assoc., vol. 83, p. 1166, 1924. See page 583.

[3] Presented to the Biochemical Society at the 6th. December meeting in the Imperial College of Science and Technology, London.

Another compound which exerts a regulatory action on portions of the body, either locally or far distant from the gland in which it is produced, is epinephrin:[1]

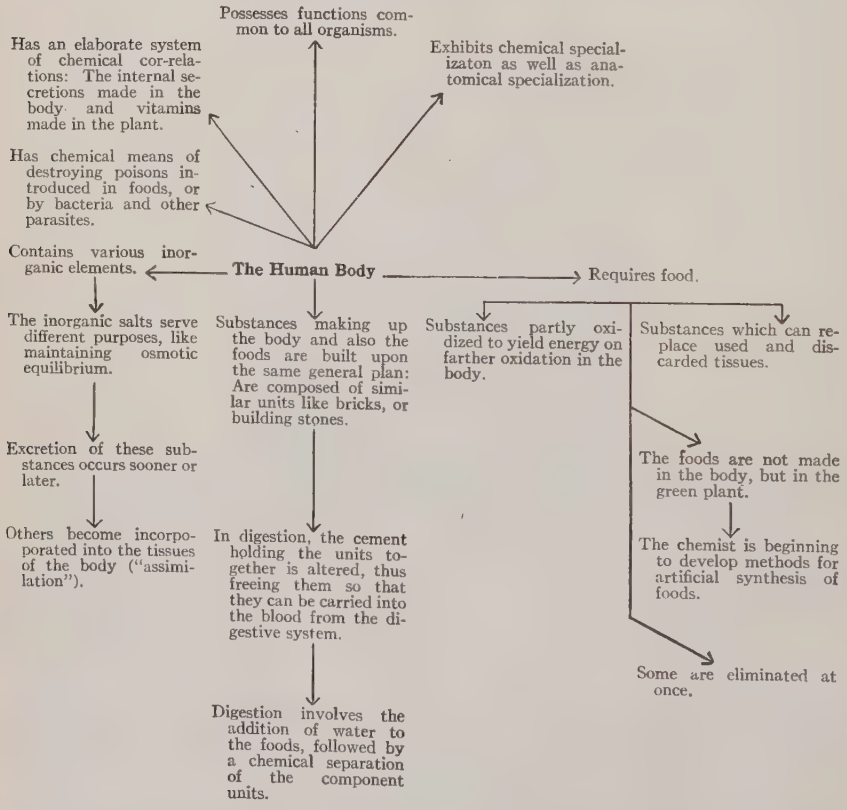


Epinephrin.

A 1:10,000 solution of this substance causes decided constriction of the smaller blood-vessels of the mucous membrane and of other tissues. Both thyroxin and epinephrin have been produced synthetically, the former by Barger and Harington (1926), the latter by Dakin (see page 583).

[1] Also called adrenin, adrenalin, and suprarenin.

GRAPHIC SUMMARY


Possesses functions common to all organisms.
Has an elaborate system of chemical cor-relations: The internal secretions made in the body and vitamins made in the plant.
Exhibits chemical specializaton as well as anatomical specialization.
Has chemical means of destroying poisons introduced in foods, or by bacteria and other parasites.
Contains various inorganic elements.
The Human Body
Requires food.
The inorganic salts serve different purposes, like maintaining osmotic equilibrium.
Substances making up the body and also the foods are built upon the same general plan: Are composed of similar units like bricks, or building stones.
Substances partly oxidized to yield energy on farther oxidation in the body.
Substances which can replace used and discarded tissues.
Excretion of these substances occurs sooner or later.
Others become incorporated into the tissues of the body ("assimilation").
In digestion, the cement holding the units together is altered, thus freeing them so that they can be carried into the blood from the digestive system.
Digestion involves the addition of water to the foods, followed by a chemical separation of the component units.
The foods are not made in the body, but in the green plant.
The chemist is beginning to develop methods for artificial synthesis of foods.
Some are eliminated at once.

Detoxication.—One of the great characteristics of the human body is the power to protect itself against dangers. Besides the morphological means of protection, such as the horny layer of the skin, there is an elaborate chemical system of rendering poisons harmless. Bacteria in the intestine produce substances which are toxic, and which are absorbed into the blood with compounds that are non-toxic. It is essential that these poisons be disposed of rapidly. This is accomplished through the liver,[1] which conjugates them with sulphuric acid[2] or with glucuronic acid, derived from glucose. Certain food substances are likewise toxic and must be detoxicated by chemical conjugation. In man, on an excessively high vegetable diet, there may be increase in toxic substances. In the herbivorous animal like the horse, there is a large excretion of these detoxicated substances, like hippuric acid. The whole problem of bacterial intoxication and how to combat it is a chemical study and a part of the general field of detoxication.

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[1] And other organs. There is a tendency in modern biochemistry to distribute the functions that once were ascribed wholly to the liver to other tissues.

[2] Sulphuric acid does not occur as such in the tissues, but what is meant is that the sulphion, in contact with certain cations, is available to perform this function.

CHAPTER IV

ENERGY PRODUCERS: THE GLUCIDS[1]

"Une fois établis, ils restent; l'interprétation humaine varie seule, comme les moyens d'observation."—*Charles Robin.*[2]

The compounds embraced by the term "glucid" are, in popular language:

The sugars: Grape-sugar, fruit-sugar, cane-sugar, malt-sugar, milk-sugar, etc.

The starches: Potato, rice, corn, and other starches.

The celluloses: Substances from which paper is made; these are the supporting structures of the cell walls of plants and are indigestible. They play an important rôle, nevertheless.

The pectins: These are the framework of the jellies; recently jellies have been made from artificial pectin, mainly derived from the apple.

Perhaps the commonest glucids are the starches. Both in the animal and the plant kingdom starch is a fundamental form of food, and also represents the stored energy derived from food. In the potato, starch is laid down in the tuber as a reserve to be drawn on in the spring. In somewhat the same way man stores starch in the liver and muscle. It is this starch which is used in producing muscle energy, as we shall explain.

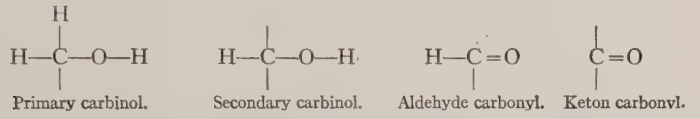
Many new glucid substances have been introduced into our dietary during the last twenty years, and additions are being made each year. The introduction of beet-sugar several years ago is an illustration of changing habits. Recently the Jerusalem artichoke has been introduced as a source of sugar (page 154). Special uses for glucids were introduced when insulin was discovered (page 20).

[1] Carbohydrates. The nomenclature is that of the International Union of Pure and Applied Chemistry. The term "carbohydrate" is misleading; it is based on the erroneous assumption that the sugars and starches have their elements arranged as carbon = n and hydrogen and oxygen as in water $(H_2O)n$. Thus glucose, having an empiracal formula, $C_6H_{12}O_6$, is resolvable into C_1 and $(H_2O)_1$. However, lactic acid $(C_3H_6O_3)$ is not a member of this group of sugars, yet its formula is resolvable also into C_1, $(H_2O)_1$. For material aid in the preparation of this Chapter the author is indebted to the writings and personal courtesies of Dr. P. A. Levene (page 23).

[2] French histologist, 1853.

Glucids are compounds of carbon, hydrogen, and oxygen. They consist of one or more simple sugars, such as glucose, bound together by the elimination of water, one molecule for each group of two simple sugars. The hydrogen and oxygen are in the form of either carbinols[1] or carbonyls.[2]

Terminology.—The suffix *-ose* has been adopted by chemists to indicate a glucid just as *-ol* indicates an alcohol, *-yde* an aldehyde, and *-ene* a hydrocarbon. Since all glucids are either aldehydes or ketons, we term them *ald-oses* or *ket-oses*.



What Kind of Energy Do the Glucids Produce?—Of the various kinds of energy which living things utilize, the most conspicuous are the energies of movement and of heat, and it is these that the glucids

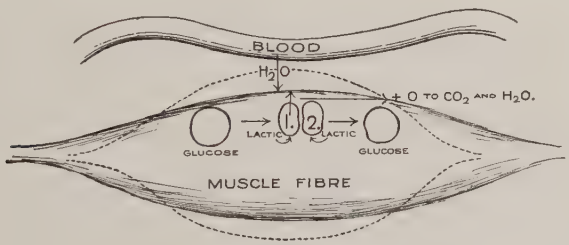


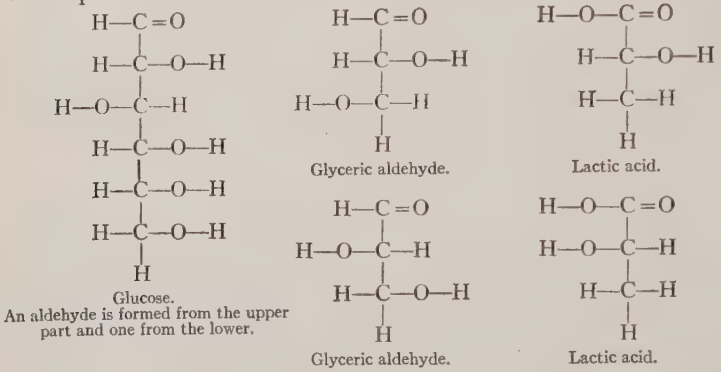
Fig. 76.—To explain the Meyerhof-Hill theory of muscle action. Glucose, at the beginning of contraction, is hydrolyzed from the store of glycogen. Six carbon glucose becomes two molecules, each, of three carbon lactic acid. A portion of the acid thus formed increases the permeability of the muscle-fiber for water; the remainder of the lactic acid becomes reconverted into glucose and glycogen. Water causes the muscle to swell (contract) and the acid is burned to CO_2 and H_2O in order to maintain the optimum temperature of the reaction. The contracted muscle is shown in the dotted lines.

produce. Little is known regarding the rôle of electricity, light, and other forms of energy in the human body. An electrical impulse accompanies the movement of muscles. We are just becoming aware of the influence which light, especially of shorter wave lengths, like ultraviolet rays of about 2200 Å, exerts upon the body, and also upon the substances used for food.[3] The energy of visible rays of light is utilized by the eye to distinguish form and color. The energy

[1] Primary and secondary alcohols.
[2] Aldehyde or keton radicles.
[3] Page 646.

of sound is used by the ear to detect the distance of objects from the human body; to appreciate rhythmic and unison music, etc.

The **manner in which the glucids are converted into energy** is interesting (Fig. 76). Glucose gives rise to two molecules of lactic acid or equivalent substance:[1]



Lactic acid increases the permeability of the muscle membranes for water, which flows into the muscle, causing greater turgidity and contraction, *i. e.*, movement. The other molecule of lactic acid by reversible reaction becomes converted into glucose again and the molecule of acid which caused the increased permeability for water is burned to afford heat necessary to maintain the optimal temperature of the reaction (Fig. 76). Glucose, then, performs a double task:

It acts physicochemically in muscle contraction, making the muscle membrane more able to absorb water.

It acts chemically, being burned, and the resultant heat maintains the temperature for the reactions in the body.[2]

It is probable that glucose alone is capable of producing energy. Pentoses are utilized to slight extent and are excreted in large amounts in terms of the quantities fed. Other hexoses than glucose that are capable of utilization (page 153, note 6) are convertible into glucose.

Structural Uses of the Glucids.—The glucids not only produce energy but also serve in various tissues as integral portions of the supporting structures:

Combined with protid they form part of the characteristic substance of the mucous slime, such as occurs in saliva and in the mucus of the mucous membrane.

Combined with protid they form a part of cartilage (gristle).

[1] The complete reactions are given on page 174.

[2] See footnote 2, page 137.

Combined with nitrogenous substances they make up the substance which the histologist calls chromatin and which is concerned with heredity and sex.

Combined with phosphoric acid they make up a compound of the general nature of the foregoing, but derived from plants and found chiefly in muscle.[1]

A glucid (milk-sugar) occurs in milk. One-half of the milk-sugar is used in making parts of the nervous tissue (medullary sheaths).

A condensation product, resembling starch, is called "animal starch" (glycogen), of which we have spoken.

A STUDY OF THE GLUCID, STARCH

As a basis for more detailed study of the various members of this important group, we shall first make a study of starch, since it is the most common of all glucid foods.

EXERCISE 1.—Examine under the higher power of the microscope[2] the various kinds of starch given you. Compare with the figures (Figs. 77–80) and be able to identify unknowns given you.

EXERCISE 2.—Using one of the preparations from the previous Exercise, add a drop of iodin solution to the granules on the slide, cover with cover-glass, and, by means of a piece of filter-paper, draw, by capillary action, some water through the opposite side; this will remove excess iodin.[3] The granules turn blue. Compare with the color given to a minute granule of dry egg-white, or casein. Distinction from protid. Explanation of the blue color: The reaction is one of colloidal adsorption.[4] Quantitatively, it may be expressed as follows:

$$\left(\frac{x}{m}\right)^{5} = k \times c$$

where k is a constant varying with the kind of substance, c the concentration of which m units are taken. The amount of iodin adsorbed[5] is x. Lanthanum acetate affects iodin in the same way that starch does. In this case, k = 2. Hydriodic acid is necessary for the reaction and for this reason the following result should be negative:

EXERCISE 3.—Into 10 mls. of distilled water in a test-tube place as much starch as will lie on a 5-cent piece. Mix until the starch is

[1] Pages 339 and 373.

[2] For table of magnification see Appendix.

[3] A drop of thiosulphate will sometimes aid in decolorizing the medium so that the granules may be seen more clearly.

[4] Page 114.

[5] See Mellor (p. 97), vol. 2, page 98 for a discussion of "starch iodide."

thoroughly dispersed through the water. Boil 50 mls. of distilled water in a small beaker and pour the contents of the test-tube into the beaker. Let the solution in the beaker boil until an opalescent suspension of starch is obtained. Cool under the tap.

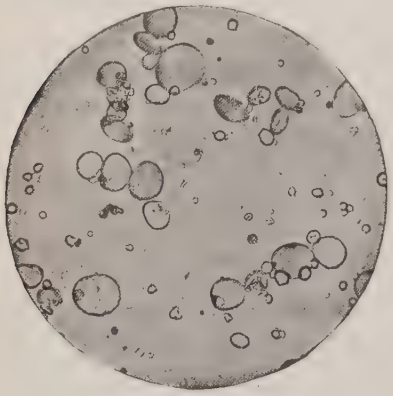


Fig. 77.—Wheat starch grains magnified 250 times. (As seen by a 6 ocular and 4 mm. objective.) (From Woodman, Food Analysis, McGraw-Hill Co., New York.)

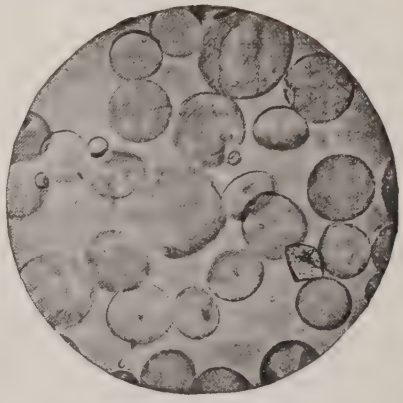


Fig. 78.—Rye starch grains. Magnification as in Fig. 77. (From Woodman, Food Analysis, McGraw-Hill Co., New York.)

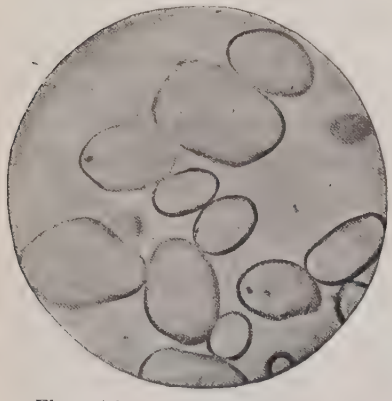


Fig. 79.—Potato starch grains. Magnification as in Fig. 77. (From Woodman, Food Analysis, McGraw-Hill Co., New York.)

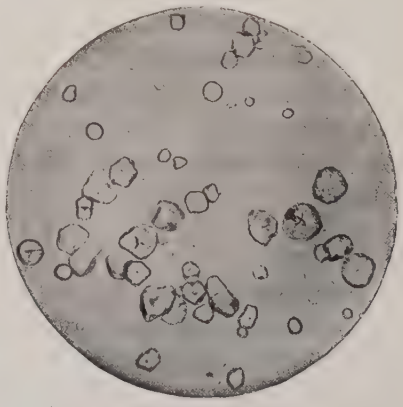


Fig. 80.—Cornstarch grains. Magnification as in Fig. 77. (From Woodman, Food Analysis, McGraw-Hill Co., New York.)

Pour 2 mls. of the solution prepared in this manner into a test-tube and add one drop of 10 per cent. NaOH solution. Mix. Add a few drops of iodin solution. Note whether color develops. Repeat,

using 1 drop of glacial acetic acid in place of the NaOH. Does the color develop?

Exercise 4.—To show that heat disintegrates the iodin-starch adsorption compound: Repeat the second part of the preceding Exercise, and after obtaining the color, gently warm the solution in the test-tube. Note that the color disappears. Cool the test-tube under the tap; the color returns.

Exercise 5.—Shake 5 mls. of the iodin solution with one volume of charcoal. Filter. Using the filtrate, add a small amount of the starch solution from Exercise 3. Do you obtain color? What was the color of the filtrate before the addition of the starch?

Exercise 6.—Decolorize a small amount of iodin solution with drops of sodium thiosulphate solution. Does such an iodin solution color starch? What would you say of the "white iodin" of commerce?

Exercise 7.—Test various sugars, cellulose (filter-paper, cotton), and pectin (commercial fruit pectin), with iodin. Note with what substances you obtain the color.

Exercise 8.—Hydrolysis of starch. To 5 mls. of the solution from Exercise 3 add 2 drops of hydrochloric acid (conc.) and place the tube in boiling water. Transfer to the concavities of a spot-plate drops of iodin solution and at intervals test the contents of the tube for the starch-iodin reaction. Note when the color becomes changed. Note when the blue color is lost. It is necessary to perform this test with cooled starch solution (see Exercise 4); therefore, remove a drop of the solution by means of a glass rod and let the liquid cool in the air before plunging into the iodin. Leave the test-tube in the bath for about five minutes after the blue color disappears and then test the contents of the tube with Fehling's solution,[1] for the presence of a simple sugar.

The Products of Starch Hydrolysis.—In Exercise 8 we found that, as time proceeded the starch underwent changes which were indicated by different colors of iodin. The first change was the appearance of a reddish color; the product of starch hydrolysis giving this color is erythrodextrin.[2]

When the color with iodin disappears, achroödextrin[3] is the product. Subdivisions of these groups of hydrolysis products of starch

[1] Page 158.

[2] Greek, *erythraios*, red, and Latin, *dexter*, right, the dextrins turning the plane of polarized light to the right, like glucose.

[3] Greek *a*, privitive, meaning without, and *chroma*, color.

may be recognized by special methods, but further detail is not warranted here. More detail is given on page 192.

Chemical Structure of Starch.—It has already been stated[1] that starch resembles other typical energy-producing or repair food substances in being built up from a number of simple units. The units from which starch is derived are molecules of glucose which gives a positive reaction with Fehling's solution and a negative with iodin. This is readily demonstrated by testing pure glucose with Fehling's solution and with iodin, or one may identify glucose absolutely by

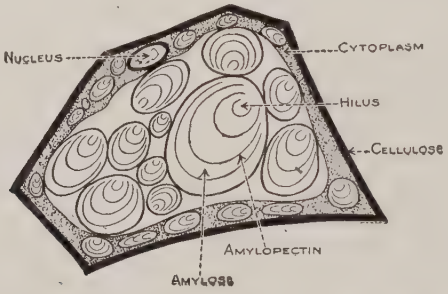


Fig. 81.—A typical starch cell. The starch is produced in the protoplasmic layer surrounding the central space, and as the starch grains develop, they intrude and obliterate the space. Each grain consists of (1) starch proper, amylose; (2) amylopectin, which protects the starch. Amylose is the nutritive starch.

means of the so-called osazone test.[2] Glucose is made into a molecule of maltose by the union of two molecules of glucose and the elimination of one molecule of water. Maltose, likewise, may be identified by means of its osazone.[3] The maltose molecules are united to form achroödextrin and this in turn composes erythrodextrin, and finally the complete molecule of starch. The best determinations of molecular weight of starch give the empirical formula as $(C_6H_{10}O_5)_7$, with a molecular weight of 32,000. However, such weights are theoretical,[4] being calculations on the assumption that colloidal starch follows the laws of gases and of solutions, and in all probability do not represent accurately the true molecular weight of the starch molecule.

Physical Structure of the Starch Grain (Fig. 81).—Starch is grouped in minute bodies or grains around a central core, the hilus. By examining a bit of potato starch under the microscope, one finds that the grains are not homogeneous throughout, but that concentric

[1] Page 135.

[2] Page 164.

[3] Page 151.

[4] Bancroft, W. D., Professor of Physical Chemistry, Cornell University, Ithaca, N. Y. See Applied Colloidal Chemistry, New York, McGraw-Hill Book Co., 1926.

Fig. 82.—Phenylhydrazin test for sugars. (From Holland, Medical Chemistry and Toxicology.)

layers of different materials exist in the grain. The true starch which turns blue with iodin, called *amylum*, *granulose*, and *amylose*, is surrounded by a protective layer of a cellulose, *amylopectin*, which does not turn blue with iodin. Cellulose, as we shall see,[1] is refractory to reagents, such as water, acids, alkali, enzymes. Granulose, on the other hand, when heated in water is readily soluble (Lintner's starch).[2] The purpose of cooking starchy foods is to cause mechanical rupture of the insoluble amylopectin layers and to make the amylose available for action of the digestive juices. However, 90 per cent. of raw potato starch is digested by man.

Identification of the Hydrolysis Products of Starch.—The dextrins are identified by their color reaction with iodin. The simpler sugars below the dextrins (maltose and glucose) are identified by the type of crystals (osazones) formed when they react with the compound phenylhydrazin ($C_6H_5.HN.NH_2$).

Exercise 9.—*Maltosazone.*—Grind together in a dry mortar as many phenylhydrazin-hydrochlorid crystals as will lie upon a quarter piece and twice as many sodium acetate crystals.[3] Using a solution of maltose, add to 10 mls. of this solution 1 ml. of glacial acetic acid and mix this solution with the phenylhydrazin—HCl—acetate mixture, warming to aid solution. Filter. Place the test-tube containing the filtrate in a boiling water-bath for half an hour. Then remove the flame[4] and let the water cool spontaneously. Crystals will appear. Follow their appearance and note the time necessary for first crystallization. Note their appearance in mass and under the lower power of the microscope, omitting cover-glass. Compare with the figures of osazones (Fig. 82). Recrystallize from hot water, dry between filter-paper, and determine the melting-point as follows: From small-bore glass tubing draw out a bulb and cut above and below the bulb so as to leave one end a funnel-shaped opening (Fig. 227). Seal the opposite end completely. With a knife blade transfer some of the crystals to the funnel, and by tapping it make them fall into the bulb. Now seal the funnel end, taking care not to melt the crystals. When the tube is cool, tap the crystals to the bottom of the bulb, invert it carefully, attach it to the bulb of a thermometer by means of rings cut from rubber tubing. Next immerse the preparation in a small beaker of sulphuric acid standing upon a wire gauze over a micro-

[1] Page 193.

[2] Lintner, C. J., German beer chemist.

[3] The acetate acts as a buffer (page 72) against the HCl, which becomes loosened from the molecule.

[4] The preparation must not be shaken.

burner. Add a crystal of KNO_3 to prevent discoloration of the acid. Increase the temperature of the acid and note carefully the melting-point of the crystals (approximately 200° C.). The Thiele tube (Fig. 227) is a desirable means of obtaining the melting-point of the osazones.

EXERCISE 10.—*Glucosazones.*—Repeat the osazone formation, using glucose. Compare the shape of the crystals with that shown in the figure (Fig. 82).

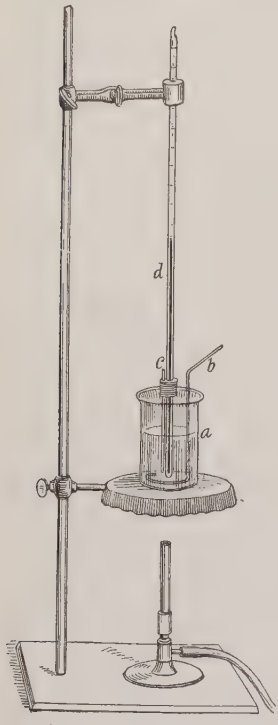


Fig. 83.—Determination of melting-point: *a*, Beaker; *b*, stirrer; *c*, melting-point tube (see Fig. 227); *d*, thermometer. (From Holland, Medical Chemistry and Toxicology.)

TECHNICAL CLASSIFICATION OF THE GLUCIDS

Let us now proceed to a more detailed study of the glucids, insofar as they are of interest to the medical student. The classification presented is based on the chemical features of the different members.

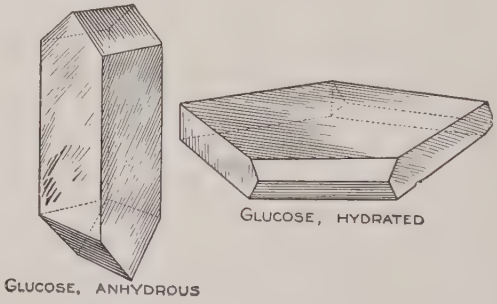


Fig. 84.—Crystals of highly purified glucose. The graphic formula for anhydroglucose is shown in Fig. 88, page 180.

Definition.—The glucids consist of the elements carbon, hydrogen, and oxygen arranged in the form of aldehydes, with the characteristic group $\begin{matrix} | \\ C{-}H \\ \| \\ O \end{matrix}$, or ketones, $\begin{matrix} | \\ C=O \end{matrix}$ and other radicles arranged as carbinols, primary $\begin{matrix} H \\ | \\ H{-}C{-}O{-}H \\ | \end{matrix}$, or secondary $\begin{matrix} | \\ H{-}C{-}O{-}H. \\ | \end{matrix}$ They are hydroxy compounds, the number of hydroxyls being one less than the number of carbon atoms. In aqueous solutions they stabilize themselves with respect to water by forming internal anhydrids, like lactons (page 182). From the fact that many, but by no means all,

have the hydrogen and oxygen proportional to the amounts of these elements in water, they are frequently called "carbohydrates."[1] While typically distributed in plants, glucids also occur in animals (glycogen in the liver; a special cellulose, tunicin, in the Tunicates,[2] etc.).

The glucids are subdivided into three groups according to the number of simple sugars, like glucose, which compose the molecule.

Monoglucids.[3]—The individual names for these sugars terminate in *-ose* (compare glucose). The molecule consists of a single simple sugar. Examples: glucose;[4] levulose;[5] galactose, in milk; mannose,[6] from manna and from the ivory nut, used for making buttons. The monoglucids may in turn be subdivided as bioses, trioses, tetroses, etc., according to the number of carbon atoms in the molecule.[7] Of these groups, the pentoses and hexoses are of primary importance to the medical student.

On oxidation monoglucids are converted into acids. Thus glucose becomes gluconic, glucuronic, or saccharic according to the degree of oxidation. On reduction, the monoglucids become alcohols; glucose, for example, becomes sorbitol.

Diglucids.—The suffix of the individual diglucids is the same as in the case of the monoglucids, *-ose*. Two simple sugars are united in the molecule in an internal anhydrid arrangement, an atom of oxygen uniting the integral parts. The arrangement is similar to that of an ether, like butylene ether.[8] The analogy extends further, because one alcohol molecule unites with another to form an ether. Glucosides

[1] Page 144.

[2] Animals lying at the base of the great vertebrate series.

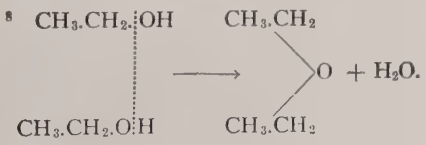
[3] Greek, *mono*, single; frequently called monosaccharids.

[4] Also called grape-sugar and dextrose. Greek *glykos*, sweet; Latin *dexter*, right.

[5] Also called fruit sugar, fructose and (recently) artichoke sugar. Latin *levis*, left.

[6] From the Oriental *manna*, food. Mannose is of interest to the physician in that it is one of three sugars (glucose, mannose and the artificial sugar glucal) which are capable of relieving the convulsions due to an overdose of insulin (see page 519). Compare their structures (page 179). For mannose see Banting, *et al.*, Amer. J. Physiol., vol. 62, page 162, 1922. For glucal see Winter, L. B. Biochem. Journ., vol. 20, page 668, 1926.

[7] Greek *bis*, twice; *tris*, thrice; *tetra*, four, etc.

[8] 

are compounds of an aldehyde (glucose) and another substance, like methyl-radicle, CH_3, or another glucid molecule. Sucrose[1] is a fructo-glucoside; maltose a gluco-glucoside; lactose, a galacto-glucoside. During digestion of the diglucids, the ether-like bond is destroyed by the addition of a molecule of water (hydrolysis).[2] Enzymes specific for the glucoside or galactoside accelerate such decompositions.

Polyglucids.[3]—The suffix is *-an*. The group consists of the starches, pectins, gums, and celluloses. The starches are glucosans; they become hydrolyzed to units identical with the diglucid, maltose. Celluloses are also glucosans. They also become hydrolyzed to diglucids. Pectins are galactosans, becoming hydrolyzed to galactose, and the unit is not a diglucid, but a triglucid.[4] A group of substances, known as hemicelluloses, occur in the coats of peas and beans and, on being fermented, produce CO_2, H_2S, and other gases, a frequent source of flatulence. These "hemicelluloses" are not true glucids, such as pentosans, hexosans, etc., but contain, in addition to pentoses and hexoses, carbohydrate acids (glucuronic and galacturonic acids; see page 157). The "hemicelluloses," therefore are allied to pectins. (See note 4.) O'Dwyer[5] recognizes two classes of "hemi-celluloses," namely, an acid-precipitable fraction containing the pentose xylose and about 11 per cent. glucuronic acid and the other fraction precipitable with alcohol, containing the pentose arabinose and much more acid, galacturonic acid. Raffinose is a triglucid composed of fructose and a galactoside, mellibiose. On digestion fructose is first separated by hydrolysis from the mellibiose and then the remaining diglucid mellibiose is hydrolyzed to galactose and glucose. Raffinose occurs in molasses. Agar-agar is a galactosan; it is prepared from certain Asiatic species of sea-weeds and is useful as a mechanical laxative. It readily absorbs water and, being indigestible, is carried to the intestine where it serves to lubricate the feces. Gums, like gum-mastic, gum-arabic, etc., belong to the polyglucids. Inulin is found in the dahlia root and affords the bacteriologist a medium for proving the presence of certain types of bacteria, which contain an enzyme capable of hydrolyzing inulin; man does not possess this enzyme. Inulin is a fructosan and is being obtained

[1] Table sugar, cane-sugar, beet sugar, saccharose.

[2] Page 136.

[3] Polysaccharids (Greek *poly*, many).

[4] An acid, d-galacturonic acid (see page 157) is probably an integral part of the molecule of all pectins.

[5] O'Dwyer, M. H. (Imp. Coll. Sc. and Tech., London), Biochem. Journ., vol. 20, page 656, 1926.

commercially from the Jerusalem artichoke; the fructose is hydrolyzed from the inulin and placed on the market in competition to cane- and beet-sugar, sucrose.[1]

CHEMICAL CHARACTERISTICS OF THE GLUCIDS[2]

The Lability of the Glucids.—The rôle played by glucids in the economy of man and animals as well as plants suggests that these substances have some peculiar property or special fitness for the work they perform. This particular function is their susceptibility to undergoing chemical change. Although the celluloses are resistant to hydrolysis and to the action of enzymes of the human alimentary tract, the individual sugars which form them are chemically very active and hence readily affected by reagents. They become oxidized easily, like all aldehydes, and it is this property that lies at the basis of several of the tests, like that of Fehling, for their detection. Their ability to become oxidized is responsible for the use of sugar in making mirrors, for sugar removes oxygen from metal hydroxides, leaving the metals, like silver, deposited upon the glass. Cupric hydroxid is reduced, especially in alkaline solution, leaving either cuprous hydroxid or oxid, or metal copper. Again, aldehydes themselves oxidize substances, that is, they become reduced, forming, like all aldehydes, alcohols of the primary series; sorbitol is derived from glucose, dulcitol from galactose, etc. Sucrose differs from other diglucids in being refractory to reagents.

Polymerization occurs on treating glucids with alkali and leads to the formation of resins. This process is the basis of Moore's test for glucids.[3] Similarly, lower aldehydes, like acetaldehyde, form aldehyde resins, insoluble in water and of dark color. The higher glucids like starch are formed from the simple monoglucids by condensation. While lower aldehydes may undergo condensation in acid solution,[4] the condensation of the glucids like formaldehyde takes place in alkaline solution. Formaldehyde in the presence of lime-water $Ca(OH)_2$ condenses into compounds of the general nature of glucose, with identical empirical formula, $(C_6H_{12}O_6)n$, but with different internal arrangement and with different optical properties.

[1] See Jour. Ind. and Eng. Chem., volume for 1924.

[2] For a brief review of the history and reactions of the glucids and a statement of the modern view see Hudson, C. S. (Bureau of Standards) A Survey of American Chemistry. New York, Chemical Catalog Co., vol. 1, page 188, 1927.

[3] Heat, in the presence of concentrated alkali.

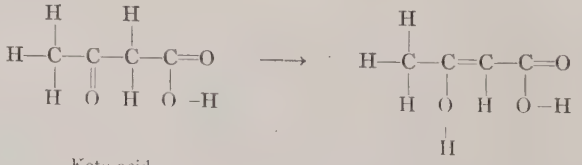
[4] Aldol-condensation.

The **instability of glucids in alkaline media** is of great importance in chemical physiology. Diabetes mellitus is caused by the inability of the organism to utilize sugar by oxidation, and it is recognized that the disease involves the intramolecular arrangement of the sugars. A change in their environment, due to disturbance of the acid-base balance, will of course affect these relations. Glucids are especially prone to undergo intramolecular re-arrangement in alkaline solution, and a characteristic change in structure is the adoption of an unstable hydroxy-form known as the enol, the characteristic radicle of which is $-\overset{\|}{C}-O-H$, as defined by Wohl and Neuberg[1] and worked in detail by Nef,[2] whose analyses of the constitution of the sugars are of primary importance. The hydrogen of the enol may be replaced by a metal or radicle, and it is probable that this is the salt formation which occurs when a sugar solution is treated with NaOH. Condensation also occurs when the sugars are heated. Sucrose heated to 200° C. undergoes condensation to caramel.

Oxidation Products of the Glucids.—We have seen that many of the characteristic reactions of the glucids are due to their ability to become oxidized readily,[3] and there is but one exception to the rule, namely, sucrose, as has just been pointed out. In order to understand the mucic acid test, which involves the oxidation of the monoglucid, galactose to mucic acid, it is well to glance at the products of oxidation of the other sugars. Moreover, in so doing acids are encountered which are of great interest not only in biochemistry, but also in pharmacology and in therapeutics.

According to the degree of oxidation, which depends upon the available oxygen, the activity of the reacting substances, or the presence of catalyzers, three categories of acids are possible:

[1] German chemists. See Ber. d. deutsch. chem. Ges., vol. 33, p. 3095, 1910. In enol formation, a keto group, $\overset{|}{C}=O$ becomes a secondary alcohol radicle, as in aceto-acetic acid:



Keto-acid. Enol acid.

The enol is an unsaturated radicle and easily undergoes changes.

[2] Organic chemist, University of Chicago, deceased.

[3] Compare page 155.

	Sugar.	Carbinol acid.	Carbonyl acid.	Dicarboxylic acid.
Bioses:[1]	CHO | CH_2OH Glycol-aldehyde.	COOH | CH_2OH Glycolic acid.	CHO | COOH Glyoxylic acid.	COOH | COOH Oxalic acid.
Trioses:	CHO | CHOH | CH_2OH Glycerose.	COOH | CHOH | CH_2OH Glyceric acid.	COOH | CHOH | CHO (Unknown.)	COOH | CHOH | COOH Tartronic acid.
Tetroses:	CHO | CHOH | CHOH | CH_2OH Erythrose.	COOH | CHOH | CHOH | CH_2OH Trihydroxy-butyric acid.	COOH | CHOH | CHOH | CHO (Unknown.)	COOH | CHOH | CHOH | COOH Tartaric acid.
Pentoses:	CHO | CHOH | CHOH | CHOH | CH_2OH Arabinose.	COOH | CHOH | CHOH | CHOH | CH_2OH Arabic acid.	COOH | CHOH | CHOH | CHOH | CHO Arabonic acid.	COOH | CHOH | CHOH | CHOH | COOH Trihydroxy-glutaric acid.
Hexoses:	CHO | CHOH | CHOH | CHOH | CHOH | CH_2OH Galactose.	COOH | CHOH | CHOH | CHOH | CHOH | CH_2OH Galatonic acid.[2]	CHO | CHOH | CHOH | CHOH | CHOH | COOH Galacturonic acid.[3]	COOH | CHOH | CHOH | CHOH | CHOH | COOH Mucic acid.[4]

Reduction Products of the Glucids.—On reduction by proper means, the sugars mentioned above are reduced to alcohols, the structural formulæ being the same as for the sugars except that the aldehyde groups becomes $-\overset{|}{C}H_2OH$. The names of the alcohols below the hexoses are adapted from those of the corresponding sugars: Glycerol from glycerose; erythritol from erythrose; arabitol from arabinose. In the hexose series the alcohols, being more familiar than

[1] Page 153.

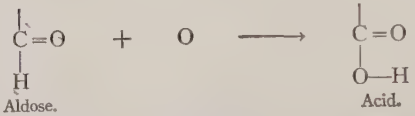
[2] Also known as lactonic acid.

[3] Galacturonic acid is responsible for the acidity of commercial pectin used in making jellies.

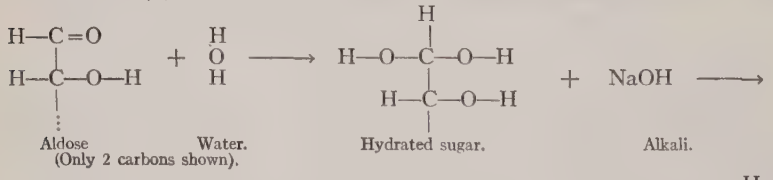
[4] Mucic acid is used in making baking powders.

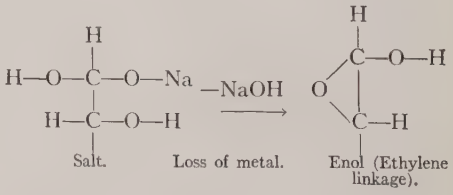
many of the others, bear more popular names: Sorbitol from glucose; dulcitol from galactose; manitol from mannose.

Reduction Tests for Glucids.—Of the many methods that have been devised to take advantage of the readiness with which the sugars reduce certain substances, only those of practical value to the chemical physiologist, and especially to the student of medicine, will be discussed here. The principle of all such tests is the oxidation of the glucid at the expense of the oxygen of the reagent. The reagent may become reduced to a compound of lower valence; or reduce to metallic form; or a new substance with lower oxygen content may be formed. Alkaline cupric sulphate[1] affords an example of the first; bismuth[2] of the second, and picric[3] acid of the third. The glucid becomes oxidized to an acid, the particular kind of acid depending upon the degree of oxidation. The reaction expressed in chemical symbols is as follows:



Exercise 11.—Arrange two test-tubes, A and B. In A place 5 mls. distilled water and add a drop of Fehling's solution[4] No. 1 Mix by rolling the tube between the palms of the hands. In B place 5 mls. of a 1 per cent. solution of glucose in distilled water and add 1 drop of Fehling's solution No. 1 as before, and mix. Now to each tube add 2 drops of 10 per cent. NaOH. Note the colloidal precipitate of cupric hydroxid in tube A and that a precipitate does not form, but a colloidal suspension remains in tube B. The reaction thus far is: (1) The NaOH converts the aldehyde radicle into an enol:



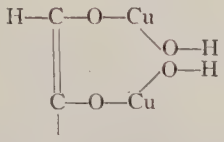


[1] Page 159.
[2] Test known as Nylander's.
[3] Page 163.
[4] See Appendix for composition.

By tautomeric rearrangement, there is formed:

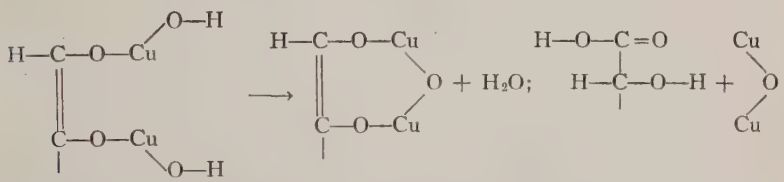
Enol.

the hydroxy-radicles of which take on cupric hydroxid by replacement of the hydrogens:



Cupric hydroxid glucid compound (dark blue).[1]

The numerous hydroxids cannot remain attached in this manner, and water is withdrawn from the two molecules of cupric hydroxid, leaving a free oxygen which oxidizes the sugar to an acid and cuprous oxid:



Dehydration and formation of lower valence of copper (cuprous oxid) and oxidation of the terminal radicle of the sugar to form an acid.

Hydrated (acid) form.

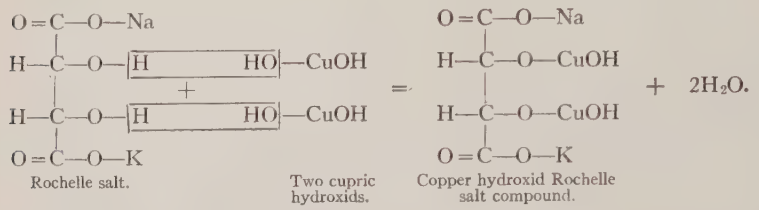
Cuprous oxid, free.

If the amount of sugar is adequate to prevent cupric hydroxid from being converted into the black hydrated form, $\begin{matrix} Cu \\ \diagdown \\ \diagup \\ Cu \end{matrix}\!\!>\!O.H_2O$, when the solution is boiled, we have *Trommer's test*,[2] which involves simply $CuSO_4$ and NaOH. By the following reaction cupric hydroxid is formed and this is reduced to cuprous hydroxid when boiled with glucose: $CuSO_4 + 2NaOH = Cu(OH)_2 + Na_2SO_4$. If, however, additional hydroxyls are introduced to hold the cupric hydroxid and to give it to the solution as needed, successful tests are almost always obtained.

[1] The description is given as if glucose acted with a single $Cu(OH)_2$ molecule, but, as Fehling first showed, it reacts with either eight or ten.

[2] Trommer, C., German chemist, published 1841. It is interesting that the original article was not considered sufficiently important to insert the full name of the chemist.

Fehling's method utilizes hydroxyls[1] from potassium and sodium tartrate (Rochelle salt):



In other respects the reaction is very similar to that given above for glucid alone.

Exercise 12.—*Quantitative Determination of Glucose by Fehling's Method.*[2]—The solution is so made that 10 mls. exactly oxidize 50 mgs. of glucose. Place the unknown solution of glucose in a burette and pipette into an evaporating dish exactly 10 mls. of a mixture of equal parts of Fehling's Solutions 1, 2 and 3. Lay the dish upon a wire gauze on a tripod over a Bunsen burner. Heat the dish and when the contents nearly boil add, a few drops at a time, the unknown solution from the burette. The appearance of a permanent yellow indicates the end-point. Since the unknown has been added until the glucose it contains just reduces the 10 mls. of Fehling's Solution in the dish and since this solution was so made that exactly 10 mls. oxidize 50 mgs. of glucose, this means that there are 50 mgs. of glucose in the volume of unknown that has been added from the burette. If the burette reading were 23.5 mls., then: 23.5 mls. $\simeq$[3] 10 mls. of Fehling's Solution $\simeq$ 50 mgs. glucose.

Then: $\frac{1}{23.5}$ of 50 mgs. gives the weight of glucose in 1 ml. of the unknown.

One hundred times this result gives the milligrams per cent. glucose.

Benedict's Solution.[4]—This is a modification of Fehling's Solution. It was especially designed by Benedict[5] for urinalysis. For solutions of pure chemicals it has no advantage over Fehling's original solution save that Benedict's reagent keeps better in solution. For any

[1] Fehling, H., von, famous German chemist, publishing seven years after Trommer, at Stuttgart.

[2] For composition of the solution see Appendix.

[3] This *sign* means equivalent to.

[4] Benedict, page 19.

[5] For Benedict's investigations concerning his method see the two following articles: Journ. Biol. Chem., vol. 3, page 101, 1907; Journ. Amer. Med. Assoc., vol. 57, page 1193, 1911.

solution involving colloidal substances, it has decided advantage; Fehling's Solution will detect glucose in 0.001 per cent. pure solution, but only 0.1 per cent. in urine; Benedict's Solution will show glucose when present in urine at 0.01 per cent. concentration. However, it requires considerable amount of glucose (0.3 per cent.) to show reduction, while the solution is hot, but after cooling, the precipitate permeates throughout the solution. It is the *bulk* of the precipitate, not the color which indicates the concentration of sugar undergoing oxidation. It is not necessary, therefore, to await precipitation to the bottom of the test-tube, for one may judge of the colloidal suspension in estimating reduction. None of the following reacts with Benedict's Solution: Chloroform, formaldehyde, chloral, uric acid and creatinin. The following are among the substances reacting with Benedict's Solution: Glucuronic acid (page 282), homogentisic acid (page 268), lactose, maltose, etc. In the urine, the discoloration seen after say 3 minutes boiling is due to urates (page 719). There is no reduction by normal urines.

Principle.—The basis of Benedict's reagent is the same as that of Fehling's (page 158). The differences have just been given.

Reagents.—See Appendix.

Procedure.—The following procedure must not be departed from: Place about 5 mls. Benedict's Qualitative Solution in a test-tube. Bring the contents of the tube to a boil; if any discoloration of the clear green solution occurs, discard the preparation but use the test-tube for the determination:[1] Add eight (8) drops (about 0.5 ml.) of the unknown solution (no more) and boil for fully, but little more than two minutes.[3] Leave the tube in your test-tube support to cool, spontaneously. Note the color of the solution both before and during cooling. The yellow color is due to the hydrated cuprous oxid $Cu_2O.H_2O$.

This solution is more dependable and useful than Fehling's:

(1) Having less active alkali (Na_2CO_3 replacing the NaOH of Fehling), there is less danger of glucose destruction before reduction. Moreover, as Folin has recently said, the citrate depresses the oxidative properties of dissolved copper compounds, so that the reaction is less sensitive.

(2) It is a single solution and therefore more convenient.

(3) Its stable qualities are superior to those of Fehling.

[1] The first boiling will oxidize any sugar present as contamination.

[2] Continued boiling will result in reactions with substances other than sugar, if such be present, as in urine.

(4) A gradation of colors ranging from olive to brick-red develops in proportion to the concentration of glucose.

(5) Tri-sodium citrate: $\begin{matrix} COO.Na \\ | \\ H—C—H \\ | \\ HO—C—COONa \\ | \\ H—C—H \\ | \\ COO.Na \end{matrix}$ is used to replace Rochelle salt because it holds the optimal amount of cupric hydroxid out of solution.

EXERCISE 13.—Set up 4 test-tubes in your rack and to A add 1 ml. of 1 per cent. glucose solution. Dilute the solution in tube A with 4 mls. distilled water, using the Ostwald-Folin pipette. Pipette 1 ml. of the resulting solution into tube B, dilute the constants of B with 4 mls. distilled water, and repeat for tubes C and D. Calculate the content of the sugar in each tube after water has been added in each case to make 5 mls. total volume in each tube.

Now in a fifth tube, E, place 5 mls. of Benedict's Qualitative Solution[1] and add 8 drops (0.5 ml.)[2] of the contents of tube A, label the tube with a piece of gummed paper, and repeat with other tubes from test-tubes B, C, and D. Leave the tubes in a boiling water-bath for exactly 2.5 minutes and then compare the colors developed. Record in your note-book the concentrations with the corresponding colors.

EXERCISE 14.—Using the diabetic urine provided,[3] make a preliminary qualitative test for glucose by the above method, recording your estimate of the percentage of sugar. Then determine the exact quantity of glucose by Benedict's Quantitative Method: The method has been used previously (page 44, Ex. 2). Fill your burette with the urine. Pipette exactly 25 mls. Benedict's Quantitative Solution into an evaporating dish and add 5 gs. Na_2CO_3 powder. Bring to a boil and add the urine carefully. The end-point is reached when the blue color is discharged, due to the formation of white cuprous thiocyanate, $Cu_2(CNS)_2$ by the reaction of reduced copper with potassium ferrocyanide ($K_4Fe(CN)_6.3H_2O$) contained in the Benedict's Solu-

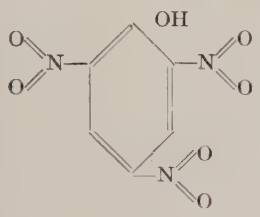
[1] The composition is given in the Appendix. If a mistake is made between Benedict's Quantitative and Qualitative Solutions different end-points are obtained. The description is for the qualitative solution.

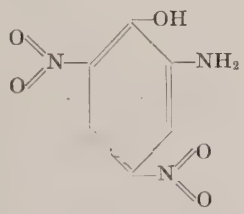
[2] For pure glucose solutions the concentration is not a vital matter, but it is when there are substances present, as in the urine, that are capable of reducing copper.

[3] If a true diabetic urine is not available, a sample of normal urine treated with 1.5 g. glucose for every 100 mls. of urine may be used, but the effect is not quite the same, owing to colloidal properties.

tion. The calculation is the same in principle as in the Fehling method, but 25 mls. of Benedict's Solution are equivalent to 50 mgs. glucose.

EXERCISE 15. Introduce 1 ml. 5 per cent. cupric sulphate solution into a test-tube and add an equal volume of 20 per cent. solution Na_2CO_3 and 2 drops of a 1 per cent. solution of glucose. Boil for two minutes. Cool under the tap and add 2 mls. of an alkaline molybdate solution.[1] Note the immediate appearance of a dark indigo-blue due to the formation of an oxid of molybdenum of uncertain composition.[2] This reaction is the basis of a quantitative method for the determination of glucose in the blood,[3] a method found in use in laboratories throughout the world. The great increase in diseases of sugar metabolism (diabetes mellitus) require such methods.

We have been considering the reduction of copper compounds by the glucid. Other substances may also be reduced. Thus picric acid,  is reduced to picramic acid,



EXERCISE 16.—To 1 ml. of 1 per cent. solution glucose in a test-tube add 5 mls. alkaline picric acid solution and boil until the solution becomes cloudy. Note the change in color during the boiling. This is the basis of Benedict's blood-sugar method.[4]

The Formation of Osazones.—We have already[5] used this method to identify the end-products of glucid hydrolysis, but we shall now consider the reactions involved. All aldehydes react with hydrazin, $H_2N—NH_2$, or its common derivative phenylhydrazin, $C_6H_5.HN—$

[1] See Appendix for constitution.
[2] Mo_2O_5, according to Klasom, German chemist.
[3] The Folin-Wu method, Chapter XVI.
[4] Chapter XVI.
[5] Page 151.

NH_2, to form compounds differing with regard to the number of reacting parts in the aldehyde. Thus, if we have a simple aldehyde, like acetaldehyde, CH_3CHO, the reaction is:

$$CH_3CH\boxed{O + H_2}N{-}NH.C_6H_5 = CH_3CH.N.NH.C_6H_5$$

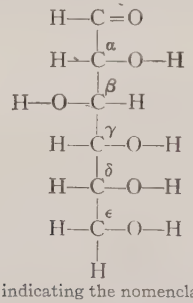
Acetaldehyde. Phenylhydrazin. Phenyl-acetal-hydrazon.

A hydrazon is formed in a mono-aldehyde, but in the case of a dialdehyde, like glyoxal,[1] there is formed a di-hydrazone, or osazone:

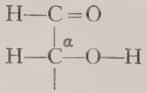
$$\begin{matrix} CH\boxed{O \quad H_2}N.NH.C_6H_5 \\ | \quad + \\ CH\boxed{O \quad H_2}N.NH.C_6H_5 \end{matrix} \qquad \begin{matrix} CH{=}N.NH.C_6H_5 \\ | \\ CH{=}N.NH.C_6H_5 \end{matrix} + 2H_2O.$$

Glyoxal. 2 phenyl-hydrazines. Phenyl-glyoxosazone.

For the higher aldehydes and ketones, the sugars and glucids in general, the principles are the same, but only the two uppermost radicles affected. Hence, whether we are considering a triose, tetrose, pentose, or hexose, we are only concerned with the ultimate[2] and penultimate[2] radicles. Hence for glucose, which, as we shall see, is



Glucose, indicating the nomenclature.[3]



we shall need but the upper two radicles[4] as given to the right of the glucose formula.

The formation of the glucosazone is represented by stages as follows:

$$\begin{matrix} CH\boxed{O \quad + \quad H_2}N.NH.C_6H_5 \\ | \\ \boxed{H}{-}C{-}O{-}\boxed{H} \\ | \end{matrix} + H_2N.\boxed{NH.}C_6H_5 = \begin{matrix} CH.N{:}NH.C_6H_5 \\ | \\ C{=}O \\ | \end{matrix} + H_2O + NH_3 + C_6H_5.NH_2.$$

Glucose. 2 phenyl-hydrazines. Hydrazone. Ammonia. Anilin.

$$\begin{matrix} CH{:}N.NH.C_6H_5 \\ | \\ C{=}\boxed{O \quad + \quad H_2}N.NH.C_6H_5 \\ | \end{matrix} = \begin{matrix} CH{:}N.NH.C_6H_5 \\ | \\ C{=}N.NH.C_6H_5 \\ | \end{matrix} + H_2O$$

Keto-phenyl-hydrazon. Phenyl-hydrazin. Phenyl-glucosazone.

[1] A biose; see page 153.

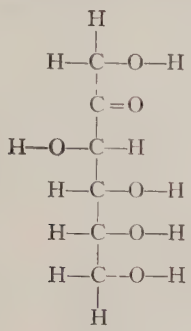
[2] Latin *ultima*, the most distant; Latin *paene*, almost.

[3] Some chemists number the carbon atoms 1, 2, 3, etc., beginning with the first or aldehyde carbon.

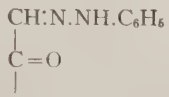
[4] The organic chemist writes the most reactive radicles first; in the case of glucose the aldehyde radicle is the most reactive, the *a* radicle next, and so on.

A di-hydrazone, or osazon, is formed in the second reaction, which is actually a third reaction, since three molecules of phenylhydrazine were used.

The Fructosazone Identical with Glucosazone.—This is evident from the comparison of the structural formula for fructose with that of the intermediate and final stages of glucosazone formation:



Fructose.



Ketone stage intermediate in glucosazone synthesis.

The sequence of stages in the synthesis of glucosazone and fructosazone is different, but the end-result is the same.[1]

Lactosazone.—It is frequently necessary for the physician to determine in a maternity case whether a reducing sugar in the urine indicates true diabetes or whether the sugar is due to lactation. Lactose and glucose may be distinguished by means of the osazones. The shapes of the crystals are different, lactosazone being fine and feathery, and also glucosazone forms much more quickly than lactosazone, owing to its lower solubility in water.

Confirmatory Test Differentiating Lactose and Glucose.—*Method of Cole:*[2]

EXERCISE 17.—(1) Place in a test-tube 2 mls. of the unknown solution which is supposed to contain both (or either) glucose and lactose and add one volume of concentrated (50 per cent.) NaOH. Boil for not less than three hours. Cool and make test with Benedict's solution; or, without cooling, add half a volume of Fehling's solution. Since strong alkali completely changes glucose into products which do not give reduction reactions,[3] while lactose is not affected, the latter, if present, gives reduction.

(2) Charcoal adsorbs lactose and permits its removal from solution and filtration. Perform the test as follows: Place 1 g. of

[1] Mannose also gives the same osazone.

[2] Cole, S. W., Professor, Trinity College, Cambridge University, England. See his text: Practical Physiological Chemistry, Baltimore, Williams & Wilkins, 1925.

[3] Page 156.

blood charcoal[1] in a test-tube and add about 10 mls. of the solution under examination. Stopper the tube, shake it vigorously, then boil, repeating this procedure at intervals several times. Filter. Filtration may be accelerated by means of a filter-pump, hardened paper, and a small Buchner funnel (Appendix). Drain the paper dry by suction and scrape off the residue (charcoal), transferring it to a small Erlenmeyer flask or large test-tube. Dilute with about 10 mls. of water and add 1 ml. glacial acetic acid. Boil gently ten minutes and filter through paper in a small funnel into a test-tube. Make the phenylhydrazin test as described for maltosazone,[2] but leave the tube in the bath for forty-five minutes and let cool as long as possible before examining for crystals. The preparation must not be agitated.

Barfoed's Acid-copper Solution.[3]—This reagent is composed of cupric acetate, $CH_3COO\diagdown\!\!\diagup Cu$, in acetic acid solution, and hence differs from other copper solutions used in sugar analysis. Whereas as small an amount as $5(10)^{-3}$ g.[4] of glucose will cause the reaction lactose in amounts smaller than $2(10)^{-2}$ g.[5] will give no reaction. Even greater concentration is required for maltose ($3(10)^{-2}$ g.[6] being required for the test).

Exercise 18.—Perform the test as follows:[7] Place 5 mls. of Barfoed's solution in a test-tube and add 1 ml. of the unknown. Leave the tube in the boiling water-bath for exactly three and five-tenths minutes and then examine it for evidences of reduction. Now dilute the solution 1:5 and repeat the procedure. If reduction is obtained before dilution, it will not occur at this dilution with ordinary solutions, like urines, unless glucose is present.

Barfoed's test as a distinguishing agent between mono- and diglucids. The statement is frequently made that Barfoed's method gives a means of distinguishing monoglucids, like glucose and fructose, from diglucids, like lactose and maltose. The basis for this assertion has just been given, where we have shown that certain quantitative relations exist between the reducing power of sugars and the reduc-

[1] The best preparation for this purpose is Merck's blood charcoal, "White Label," purified by acid. Other brands are on the market and may be useful for this purpose.

[2] Page 151.

[3] The reagent is described in Appendix. Barfoed, C. T., Swedish physician of the 19th century.

[4] 0.0050 g.

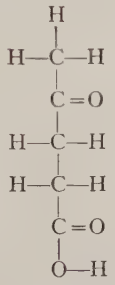
[5] 0.02 g.

[6] 0.03 g.

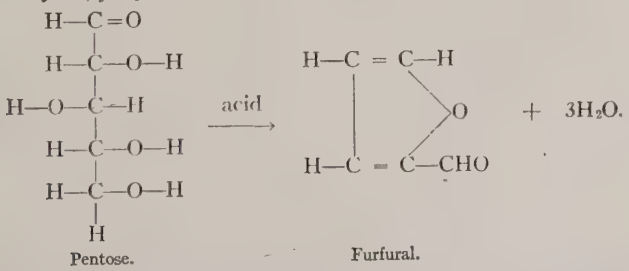
[7] Hinkel, F. C., and Sherman, H. C. (Columbia University), Jour. Amer. Chem. Soc., vol. 29, p. 1744, 1907.

tion of copper acetate. In larger amounts it is impossible to distinguish the several sugars, but in smaller quantities it is possible to so adjust the volumes and concentrations of both sugar and copper that an effective test is obtained.

The Effect of Acids on Glucids.—The simple sugars are more stable in acid than in alkaline media, but nevertheless do undergo changes in acid media. The fact that an acid copper solution is reduced by glucose, as in Barfoed's method, suggests that the effect of acid may, in some respects, be similar to that of alkali. When glucose is boiled with mineral acid, like HCl, a characteristic product is the five-carbon gamma keto-acid, levulinic acid



which is responsible, probably, for the reduction; yet other substances, like formaldehyde and CO_2, which accompany the formation of levulinic acid, may be factors in this reaction. Continual boiling of any of the glucids with mineral acid leads to the production of dark brown or black substances of uncertain composition; some of these are doubtless condensation products. The boiling of pentoses[1] with acid gives rise to products which differ from those resulting from similar treatment of hexoses (glucose). The characteristic product of the pentoses is a cyclic aldehyde, *furfural*[2] (using l-xylose):



Pentose. Furfural.

EXERCISE 19.—**Detection of Pentose.**—(1) *By distillation after acid treatment.*—Dip into a furfural solution a piece of filter-paper

[1] Five carbon sugars; see page 157.

[2] Furfuraldehyde, or "furfurol;" but since it is an aldehyde and not an alcohol, it must not be called the latter term, which has the alcohol suffix, *-ol*.

which has been treated with anilin acetate.[1] In the presence of furfural the paper turns crimson. This is the principle of the following exercise:

In a 500 ml. distilling flask place a handful of bran and 300 mls. of dilute hydrochloric acid (1 volume of concentrated acid to 2 volumes of water). Connect the flask to a Liebig condenser provided with a receiver. Heat the contents of the flask to boiling and distil until about 50 mls. of the distillate have been collected. Note the characteristic odor of the furfural in the receiving flask. What is the source of the furfural? Test one portion of the distillate with aniline acetate paper, and another with Bial's reagent. To a third portion (10 mls.) add a few crystals of phloroglucinol. To a fourth portion, add half a volume of benzene (C_6H_6) and, after stoppering the tube, shake it vigorously for about 3 minutes. Let stand about 15 minutes. By means of a pipette, transfer 2 mls. of the benzene layer to a dry test-tube taking care that none of the aqueous layer accompanies the benzene. Add 4 mls. of the McCance benzidin reagent.[2] Leave 10 minutes. Note the red-violet color. Note the change in color of the solution from the initial light yellow to dark yellow, red, green, purple and finally almost black. On standing a purplish black precipitate forms. This reaction is the basis of the gravimetric method for the estimation of pentoses and pentosans in feeding stuffs.

(2) *Phlorogiucinol test* (*Tollens'*[3] *Test*).—Principle.—Condensation of an aromatic substance with an aldehyde in acid solution.[4] Procedure: To about 5 mls. of pentose solution add one volume of concentrated hydrochloric acid and as much phloroglucinol[5] as the size of a pea. Place in a boiling water-bath. After a time a reddish color develops. If the solution is cooled and filtered, the residue dis-

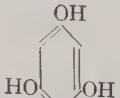
[1] Not acetanilid. Anilin acetate is $C_6H_5.NH_2.CH_3.COOH$ and is prepared by the method given in the Appendix.

[2] McCance, R. A. (Kings College, London). Biochem. Journ., vol. 20, page 1111, 1926. The reagent is as follows: Grind up 0.5 g. benzidin-sulphate with a few mls. of an alcohol-acetic acid solution (equal parts of "absolute alcohol" and glacial acetic acid). Then pour into a 100 ml. volumetric flask and make to the mark with the alcohol-acetic mixture.

[3] Tollens, B., German chemist.

[4] This is an example of many such syntheses, utilized to detect a particular agent. See pages 116, 170, and 474 (Pettenkofer's Test).

[5] Phloroglucinol:



Phloroglucinol.

solved in alcohol and examined by means of the spectroscope, a characteristic band or series of bands are found in the region of the yellow[1] and the yellow-green.[2] The heated solution itself may show bands without filtering.

(3) *Orcinol Test* (Bial[3]).—In a test-tube boil about 5 mls. of the solution to be tested and, while boiling, add the reagent drop by drop; a bright green solution will result if pentose is present. Shake up the solution with amyl-alcohol, which dissolves the green substance and hence concentrates the color.

The Color Reactions of the Pentoses.—It is not known definitely what substance or substances are responsible for the phloroglucinol reaction, the anilin acetate test and other pentose procedures, but furfural is to some extent a participant in the reactions. Its intermediate forms may likewise be responsible. While certain substances known to contain pentoses give reactions similar to those of the pure pentoses when boiled with acid, the spectroscope fails to confirm the belief that the colors are due to furfural.

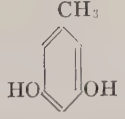
Quantitative Test for Furfural.—*Colorimetric Method of Youngburg and Pucher.*[4]—Principle: Reaction between furfural and anilin acetate, as described on page 168, the color being used for colorimetric comparison with that of a known solution.

Procedure.—A known quantity of the pentosan[5] is boiled with mineral acid for several hours and the furfural distilled into a receptacle at the end of a Liebig condenser. Then pipette 2 mls. of the distillate, which has been carefully measured, into a 100-ml. volumetric cylinder, add 2 drops of 0.5 per cent. ethanol solution of phenolphthalein as indicator and into two similar cylinders pipette, respectively, 1 ml. of the weaker standard solution and 1 ml.[6] of the stronger solution.[7] Then[8] to all three tubes add successively drop by drop 50 per cent. NaOH solution until a permanent pink is obtained. Then add to each cylinder 0.5 ml. anilin (Mohr pipette) and 4 mls. acetic

[1] The D-line; about 6000 Å.

[2] The E-line; about 5500 Å.

[3] Bial, M., German physician.



[4] Youngburg, G. E., (Prof. Biochem., Univ. of Buffalo) and Pucher, G. W. (Buffalo General Hospital, Buffalo, N. Y.), Jour. Biol. Chem., vol. 61, p. 741, 1924.

[5] Substance composed of pentoses and from which pentose must be freed by hydrolysis.

[6] Containing 0.02 mg. furfural per 1 ml. of solution in water.

[7] 0.05 mg. furfural per 1 ml. solution.

[8] During the process of neutralization the cylinders must rest in cold water.

acid. Dilute to 100 mls. with distilled water and mix. Let stand in a dark place for fifteen minutes. Compare in a colorimeter.[1]

The calculation is made on the following principle:[2]

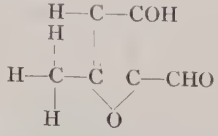
$$\frac{\text{Reading of the standard in mm.}}{\text{Reading of the unknown in mm.}} = \frac{\text{Concentration of unknown.}}{\text{Concentration of standard.}}$$

Effect of Acids on Diglucids and Polyglucids.—The application of acids to diglucids and polyglucids causes hydrolysis which destroys the ether-like linkage of the component simple sugars. This will be discussed later (pages 189 and 428).

Distinguishing Test for Ketoses.—Levulose appears in the urine in cases of metabolic errors involving the hexoses[3] (diabetes mellitus). Pure levulosuria is a rare condition. However, a functional test for hepatic efficiency, dependent upon the liver's power to convert fructose into glycogen, has been described. This power is interfered with when the liver is diseased, as in cirrhosis, and the physician is called upon to distinguish levulose from other hexoses. The test herewith given is frequently spoken of in biochemistry and in clinical medicine as a test for levulose, but it is rather a test for any ketose, whether levulose or another ketose. It is essential to perform the test exactly according to directions, for slightly positive reactions may be obtained from aldoses.

Exercise 19a.—**Selivanoff's[4] Test.**—Place 5 mls. of the reagent[5] in a test-tube and add a few drops of the suspected fluid. Heat the contents of the tube to boiling. A red color and brownish precipitate form if levulose is present. The precipitate may be filtered off and taken up in strong alcohol for the purpose of spectrum analysis; the spectrum is similar to that of the phloroglucinol reaction.[6]

What is responsible for the Selivanoff reaction? The effect of acid on any hexose, but especially on those which are ketoses differs from that on pentoses and leads to the formation of two characteristic groups, namely, levulinic acid[7] and a secondary alcohol derived from furfural, known as methyl-hydroxy-furfural:



[1] For the description of a colorimeter and method of use see Chaps. XV, XVI.

[2] This is the general formula of Beer for colorimetric calculations. Beer, A. Poggendorf: Annalen der Physik., vol. 86, page 78, 1852.

[3] It is supposed that tautomeric (Greek *to auto*, the same) changes occur, glucose and levulose being interchangeable.

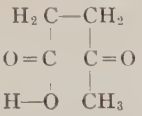
[4] Selivanoff, Th., Serbian-German chemist, publishing in 1887.

[5] See Appendix for the reagent's composition.

[6] Page 168.

[7] Page 171.

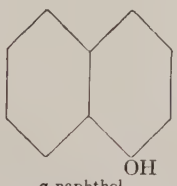
It is this substance which condenses with the resorcinol of Selivanoff's solution, to form the colored substance of the test.[1] Levulinic acid, built somewhat like a thiophen:



may become condensed with the above compound to form a furfural-levulinic acid, which may be partially responsible for the reaction.

Molisch Reaction: A General Test for the Presence of Glucid. To determine whether any form of glucid is present in a solution the following test is used. It is applicable to pure sugars, starches, or celluloses, and also to glucids which are a part of other compounds, such as the protid-glucose compounds and the phosphoric esters of glucose;[2] all of which will be described later.

The chemical principle of the Molisch reaction[3] is similar to that of the Selivanoff reaction, namely, a condensation of an aldehyde in the presence of another substance to form a characteristic colored substance. The special chemical agent in the Molisch solution is alpha naphthol:



α-naphthol.

Exercise 20.—Place 5 mls. of the unknown glucid solution in a test-tube. Mix with 5 drops of the solution of α-naphthol.[4] Layer carefully with concentrated sulphuric acid,[5] and at the zone of junction a violet ring indicates a positive reaction.

The Special Chemistry of Fructose (Levulose).[6]—It has been found[7] that fructose can be obtained in large quantities from the

[1] Compare the reaction and note, page 170.

[2] Mucin, of the saliva, is an example of the protid-glucid compound (page 320) and hexosephosphoric acid esters of the second (page 176). The latter are, at the present time, affording great interest in relation to the question as to how the glucids are utilized in the body.

[3] Molisch, H., Austrian chemist, publishing 1886.

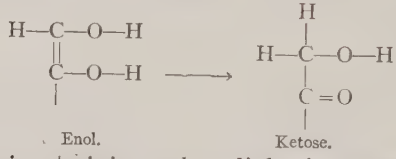
[4] See Appendix for composition of the solution.

[5] These strong reagents should never be sucked from a bottle through a pipette by means of the mouth. Either use a rubber-bulb dropping bottle, or, holding the stoppered bottle over the test-tube, carefully remove the stopper and permit a drop to fall.

[6] For a method of determining fructose in a mixture of other substances see Campbell, W. R. and Hanna, M. I. (Toronto), Jour. Biol. Chem., vol. 69, page 703, 1926.

[7] Department of Agriculture, Washington, D. C.

Jerusalem artichoke,[1] which has been known for many years to contain inulin, a fructosan.[2] This plant can be grown throughout the world, whereas sugar-cane can be raised only in warm climates. Fructose can be crystallized to resemble sucrose and will become a competitor of our ordinary table-sugar. The student of medicine should become familiar with its chemistry and physiology. Chemically, fructose is a ketose. The formula is given on page 176 where it is shown as straight-chain compound; on page 189, however, where it is shown as a component of sucrose, it is given as a γ-sugar to show its highly reactive nature. In sucrose it is linked as a gluco-fructoside with glucose. The following reactions show how fructose is converted into glucose in the animal body (refer to enol, page 159);



Enol. Ketose.

When fructose is administered to diabetics, a negligible increase in tolerance is believed by clinicians to take place. However, as Folin and Berglund[3] have shown, administration of fructose is accompanied by diarrhea and secondly, since the organs readily absorb sugars of whatever kind up to a saturation point (compare MacLeod's expression "vacuum for sugar"), fructose is absorbed but not necessarily used.

The behavior of fructose in yeast fermentation—a process about too be discussed is frequently misunderstood. One method of obtaining fructose is to subject a mixture of glucose and fructose, as in cane-sugar or in "invert-sugar" to yeast fermentation whereupon glucose disappears first, leaving fructose. Individually, however, glucose and fructose are fermented equally and in a mixture, ultimately, both are destroyed. We shall see that fructose plays an important rôle in the metabolism of sugar both in formentation and in the body, owing to its reactive nature (pages 176 and 189). It is being used as a part of invert sugar, "fondant,"[4] etc., in confectionery. Altogether the American family will use greater quantities of fructose.

[1] To be distinguished from the "artichoke" commonly seen in the fruit stores. The name is a corruption of the Italian name for sunflower (girasole). The Jerusalem artichoke is edible only in the tubers which grow underground and act as storehouses of the starch-like substance, inulin.

[2] For the meaning of the suffix see page 154.

[3] Folin, O. and Berglund, H. Jour. Biol. Chem., vol. 51, page 213, 1922.

[4] This is the technical name for a mixture of glucose and sucrose. An enzyme capable of hydrolyzing the sucrose of fondant is added, which produces free fructose from the sucrose.

FERMENTATION

When the methods of detecting lactose were discussed[1] one of the most useful was intentionally omitted because it involved a discussion of fermentation. This is the method of Mathews:[2] Using 50 mls. of the diabetic urine[3] suspected of containing both lactose and glucose, make Benedict's quantitative determination.[4] Record your result; this gives the total reducing power of the urine. Then subject 20 mls. of the urine from the original sample to fermentation by placing them in a 200 × 20 mm. test-tube and adding half a cake of fresh, compressed yeast. Mix thoroughly and leave at 42° C. for one hour. Agitate the contents of the tube at intervals and have the tube inclined so as to permit the release of bubbles of gas, CO_2, which come off continually if glucose is present. Filter, using a fluted (Fig. 218) filter, and again determine the reducing power by means of Benedict's quantitative method on the filtrate. The difference between this figure and the total reduction, first obtained with Benedict's quantitative solution, is the glucose concentration. The glucose has been fermented by the yeast, but lactose, if present, remains intact, for yeast does not ferment it.[5] Identify lactose by the osazone. Corroborate the statements regarding the fermentation of lactose and glucose, and extend the experiment to galactose, maltose, fructose, sucrose, starch, a pentose, like arabinose, or xylose, to cellulose (filter-paper), and to pectin (commercial apple pectin).

The Chemistry of Fermentation.— The term "fermentation"[6] is a group name for the action of enzymes[7] accelerating oxidations, reductions, hydrolyses, and other chemical reactions in organic compounds, like sugars, leading to end-products such as alcohol, organic acids, aldehydes, CO_2, H_2O, H_2, etc. We shall discuss principally alcoholic fermentation of the sugars because the chemistry involved is somewhat similar to that of the normal metabolism of sugar in the

[1] Page 165.

[2] Mathews, A. P., Professor of Biochemistry, University of Cincinnati, Cincinnati, Ohio. See Preface. See also the text: Physiological Chemistry, New York, William Wood & Co., 4th ed., 1925. (See Fig. 158.)

[3] Or a synthetic urine made by adding fructose and glucose.

[4] Page 44.

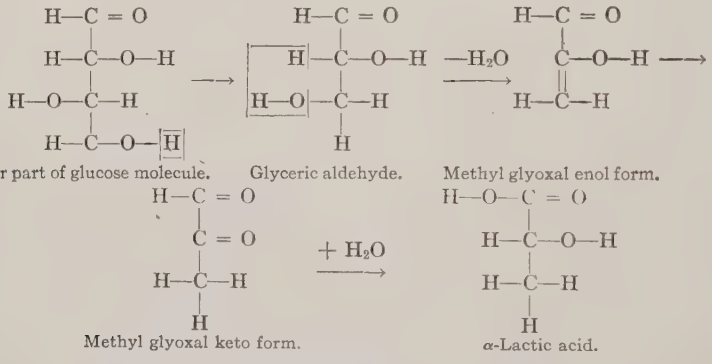
[5] Under the conditions of the experiment, compare page 172 where the fermentation of fructose alone and mixed with glucose is discussed. Any yeast capable of fermenting galactose can ferment, also, mannose, fructose and glucose if the sugars are isolated from others.

[6] Latin *ferveo*, to boil; *fermentum*, yeast; that is, the substance which causes solutions to form gaseous exudations or ebullitions seen in fermenting concoctions.

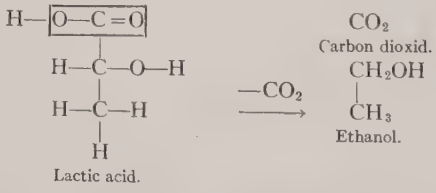
[7] Greek *en*, within, and *zyme*, yeast.

human body.[1] However, since lactic acid fermentation affords a simpler view of the processes of fermentation, as a chemical reaction, it will be considered first.

Lactic Acid Fermentation.- The hexose is composed as the term[2] implies, of six carbon atoms united into primary and secondary alcohol and aldehyde or keton radicles. The structure is shown on page 164, where the atoms are indicated by Greek letters, α, β, γ, δ, and ϵ. On page 146 we have also shown the decomposition of glucose into two molecules of lactic acid. It is in the region of the gamma (γ) carbon atom that the chain disrupts. In the process of fermentation lactic acid is split from the hexose.[3]



The same transformation takes place in the lower half of the hexose molecule; thus two molecules of lactic acid arise from a single molecule of hexose. Finally, lactic acid is converted into ethanol by decarboxylation:



The end-products are ethanol and carbon dioxid. The bubbling off of the gas is "fermentation." The process of decarboxylation is common to the lower forms of life, but uncommon in man, for in many cases it involves the production of extremely poisonous compounds, especially where protids are concerned.[4] Lactic acid fermentation is

[1] Page 176.

[2] Greek, *hex*, six, and *-ose*, suffix accepted as meaning sugar.

[3] In the case of the souring of milk lactose is hydrolyzed into glucose and galactose and lactic acid is derived from both.

[4] Page 260.

brought about by bacteria of certain species, like Bacillus acidophilous, which is prepared in cultures, embedded in candy, which, when taken into the alimentary tract of man, is digested and the bacteria freed; they become fixed in the intestine and aid in regulating the flora,[1] and in eliminating excessive numbers of undesirable bacteria. "Salt-rising" bread utilized the same species of bacteria known to produce gas disease.

Alcoholic Fermentation.[2]—The principles of lactic acid fermentation are similar to those of alcoholic fermentation by yeast. Besides methyl glyoxal, lactic acid, ethanol, and CO_2, other substances of intermediate nature have been identified in the fermentation by

Fig. 85.—Fermentation tube. For mounting, when in use, see Fig. 86.

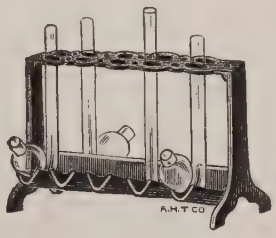
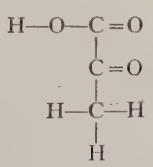
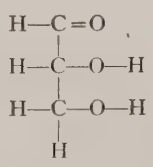


Fig. 86.—Fermentation tubes in a test-tube rack.

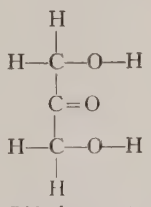
yeast. Of these, pyruvic acid,[3] glyceric aldehyde,[4] and dihydroxy aceton are particularly characteristic. All of these substances bear hydroxy- or ketone radicles which indicate that they are substances of incomplete combustion:



Pyruvic acid.



Glyceric aldehyde.



Di-hydroxy-aceton.[5]

[1] The term (Latin, flower) refers to the members of the vegetable kingdom growing in a certain region. Here it means the kind of bacteria.

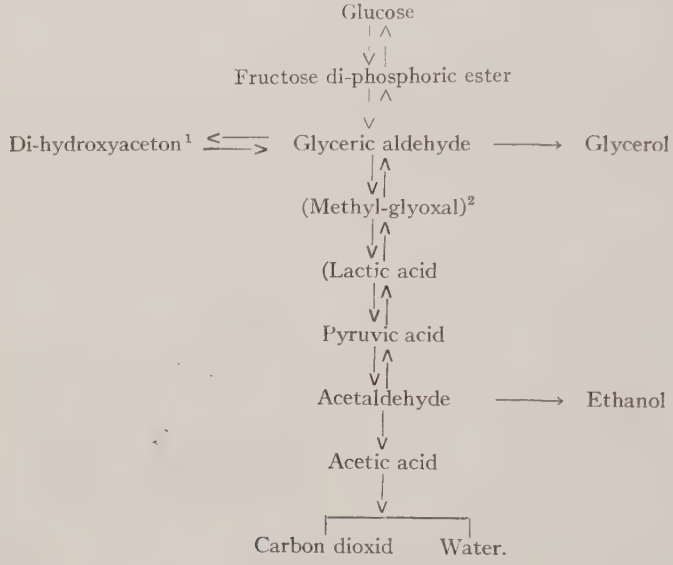
[2] For an account of fermentation involving the modern point of view see Nord, F. F. Chem. Reviews, vol. 3, page 41, 1926.

[3] The acid of which the aldehyde is methyl glyoxal.

[4] The aldehyde the alcohol of which is glycerol.

[5] The fact that di-hydroxyaceton does not ferment as quickly as glucose rather eliminates this substance as an intermediate product in fermentation. However, a mixture of glucose and di-hydroxyaceton oxidizes much more quickly than either separately.

The relation between these various substances is represented by the following table: The amount of lactic acid in fermentation mixtures by yeast leads to the question whether this acid is an intermediate product in alcoholic fermentation. Lactic acid is included in the following scheme as a possibility:



THE METABOLISM OF GLUCIDS

The foregoing table serves, in a general way, to indicate the sequence of stages in the metabolism of glucose in the body, but certain factors have to be modified. In both cases there is an intimate association of glucose and phosphoric acid. This has been explained in various ways, but some such scheme as the following equation may represent the relation:

$$\underset{\text{Glucose.}}{2C_6H_{12}O_6} + \underset{\text{Phosphoric acid.}}{2H_3PO_4} \longrightarrow \underset{\text{Fructosediphosphoric ester.}}{C_6H_{10}O_4(H_3PO_4)_2} + \underset{\text{Carbon dioxid.}}{2CO_2} + \underset{\text{Water.}}{2H_2O} + \underset{\text{Ethanol.}}{2C_2H_5OH}$$

The rôle of the acid is unknown. It is cleaved from glucose during the utilization of glucose in the body, and when insulin is administered, it is synthesized.

[1] Campbell, *et al.*, have shown that di-hydroxyaceton is not in the line of metabolism of glucose. However, there is relief of insulin hypoglycemia by means of di-hydroxyaceton. Campbell, W. R., Fletcher, A. A., Hepburn, J. and Markowitz, J. Jour. Biol. Chem., vol. 67, page lvii (Proc.), 1926. Also Kermack, W. O., Lambie, C. G. and Slater, R. H., Biochem. Journ., vol. 20, page 486, 1926. The latter authors, believe di-hydroxyaceton to be directly utilizable in the body, both normal and diabetic and that it need not be converted into glucose first.

[2] The rôle of methyl-glyoxal, as well as that of lactic acid, is questionable.

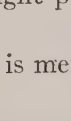
Secondly, the similarity if not identity of alcoholic fermentation and sugar utilization in the body is made evident by the fact that those sugars that are utilized in the body of a diabetic when insulin is given are those and only those that are fermentable by yeast.[1] On a later page we shall discuss these matters in greater detail.

OPTICAL RELATIONS OF THE SUGARS

Substances which have been or are connected with living things exhibit optical activity, that is, possess the power of rotating polarized light. This statement may be challenged by mentioning substances which are known not to have been connected with life and which nevertheless exhibit this power, an example of such substances being quartz, but in such instances there is little correlation with any other factors, while in the case of optical activity in substances in living things there is a constancy. Almost all the glucids utilized in the organism are dextrorotary, while the protids are practically built upon a levorotatory basis. A specificity of enzymes follows this phenomenon.

What Causes Optical Activity?—The answer to this question is the result of studies by Pasteur[2] who, as a boy, detected the difference between dextro- and levorotatory crystals by means of certain facets on the crystals of d-tartaric and l-tartaric acid, and found that racemic[3] tartaric acid crystals were intermediate between the two, and may be considered as equal mixtures of d-rotatory and l-rotatory acids. Now we know that there is a definite relation between chemical constitution and physical structure, due to LeBel and van't Hoff.[4]

The Asymmetry of the Carbon Atom.—The students of chemistry just mentioned explained optical activity by assuming that the carbon atom possessed four equivalent valencies, each directed toward the inner angles of a tetrahedron (Fig. 87). If these affinities are satisfied by four similar elements or radicles, light passing through the substance is unaffected. Such a substance is methane



If, on the other hand, all four are attached to four *different* substances, the atom becomes asymmetrical and light passing through will

[1] See Herring, P. T., Irvine, J. C., and Macleod, J. J. R. (for Macleod, see page 20; Irvine is Principal of St. Andrews University, Scotland), Biochem. Jour., vol. 18, p. 1023, 1924.

[2] Pasteur, L., French savant.

[3] Optically inactive.

[4] LeBel, J. A., living in France; van het Hoff, J. H., Dutch chemist, deceased.

become altered. Polarized light, that is, light in one plane, is affected by causing it to become displaced in the direction of that portion of the molecule which is preponderatingly asymmetrical. A substance like $1-\overset{\overset{2}{|}}{\underset{\underset{4}{|}}{C}}-3$, where the numbers indicate different substances or radicles, will cause a rotation of the light. If the spin is toward the right, as seen in a polariscope,[1] the substance is called "dextrorotary"

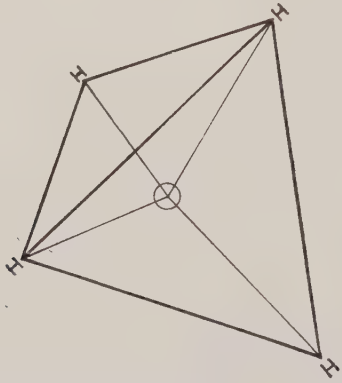


Fig. 87.—Model of a carbon atom having its four valencies attached to a hydrogen, each making CH_4 (methane). The bonds are of equal value, the carbon mass being assumed to occupy the center of a regular tetrahedron.

and "levorotatory" if the spin is toward the left. As a standard Emil Fischer[2] recommended dextrorotatory glucose[3] to which he gave a structural formula with the primary and secondary alcohols and the aldehyde group placed in a certain way to indicate the optical properties:

$$\begin{array}{r|l} H & O \\ H & OH \\ HO & H \\ H & OH \\ H & OH \\ H & OH \\ & H \end{array}$$

d-Glucose, showing the preponderance of hydroxyls on the right to indicate dextrorotary properties. The line represents carbon atoms.

Isomeres.—Any substance having the general configuration of d-glucose is known as an isomer[4] of glucose, and according to the

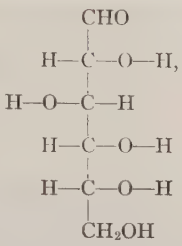
[1] Page 184.

[2] Emil Fischer, foremost German organic chemist, University of Berlin, died 1919.

[3] See page 164 for structural formula and notation of carbon atoms.

[4] Gr. *isos*, same, and *meros*, part.

empirical findings of van't Hoff, it is possible to have as many isomeres as there are asymmetrical carbon atoms; but, in addition, since it is possible to make mirror pictures of each isomer, there are as many isomeres possible as 2^n carbon atoms that are asymmetrical. The following formulæ illustrate this point. They are abbreviated, only the right side being given; that is, d-glucose,



becomes OH, H, OH, OH, giving the hydrogens and hydroxyls on the right side of the asymmetrical carbons:

Aldohexose series:

d-Glucose	OH, H, OH, OH	l-Glucose	H, OH, H, H
d-Mannose	H, H, OH, OH	l-Mannose	OH, OH, H, H
d-Galactose	OH, H, H, OH	l-Galactose	H, OH, OH, H
d-Talose	H, H, H, OH	l-Talose	OH, OH, OH, H
d-Idose	OH, H, OH, H	l-Idose	H, OH, H, OH
d-Allose	OH, OH, OH, OH	l-Allose	H, H, H, H
d-Altrose	H, OH, OH, OH	l-Altrose	OH, H, H, H
d-Gulose	H, H, OH, H	l-Gulose	OH, OH, H, OH

Of these, three, with their isomeres, occur in nature (the first three); of the others, all the d-forms are known, but of the l-isomeres, l-allose and l-altrose are unknown.

Ketohexose series:

d-Fructose	H, OH, OH	l-Fructose	OH, H, H
d-Sorbose	H, OH, H	l-Sorbose[1]	OH, H, OH
d-Tagatose	H, H, OH	l-Tagatose[2]	OH, OH, H

The ketone series have but three asymmetrical carbon atoms, and hence $2^n = 8$, so that out knowledge is incomplete concerning the ketohexoses.

The reader is warned that the designations "*d-*" and "*l-*" have no significance with regard to the optical relations, but simply mean that taking a certain arrangement to represent a known sugar, the other configurations follow. Thus, the sugars, glucose, and mannose

[1] Note that l-sorbose resembles d-glucose; Hudson (American chemist) believes that the designation l-sorbose should become d-sorbose for this reason.

[2] Unknown.

among the aldoses, and fructose among the ketoses, have the property in common of being fermented by yeast; and the similarity of the last three carbon atoms (H, OH, OH) is meant to indicate this similarity along with other common properties.

These formulæ, however, present certain difficulties—some of the most important characteristics are not represented. Thus, a formula such as that for d-glucose does not conform to an organic chemist's idea of the activity of such a compound; glucose is not as active as the formula, with the terminal aldehyde, would imply. In

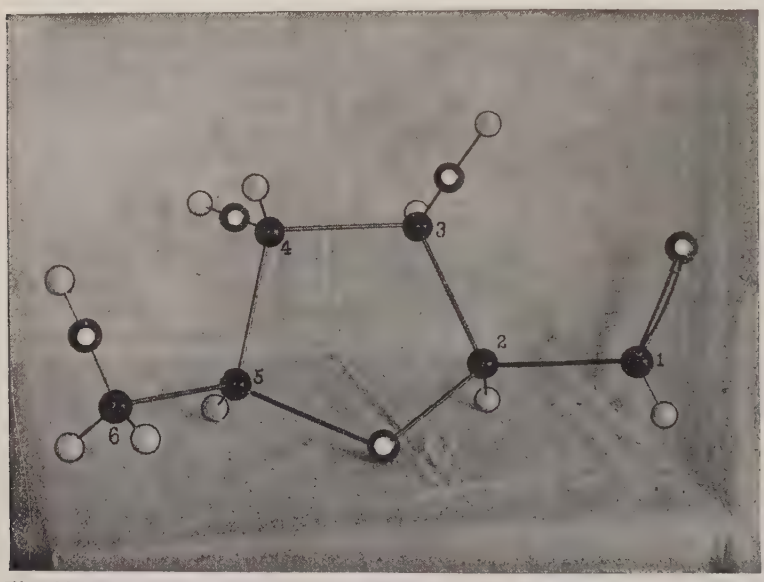
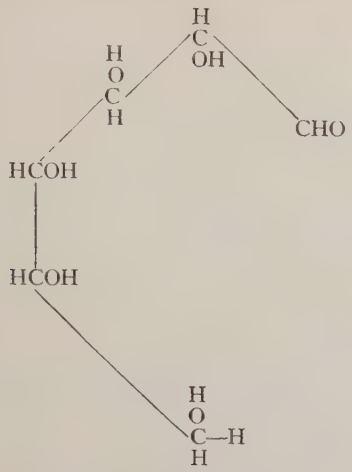


Fig. 88.—Levene's model of a form of glucose (2, 5-anhydro-glucose), designed to show (1) the cyclic anhydride ring and (2) the free aldehyde (extreme right). Carbon atoms in black; hydrogen in white and oxygen black with white centers. The presence of the free aldehyde indicates the great activity of the substance. (Jour. Biol. Chem., vol. 59, p. 137, 1924.)

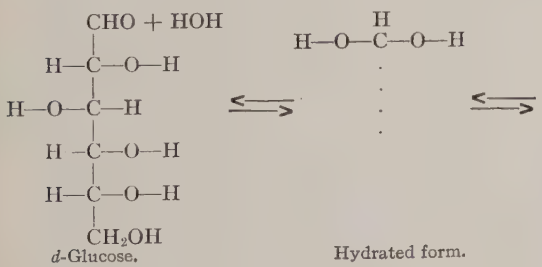
order to meet this and other difficulties the chemist has resorted to the same designation that he uses in the aromatic series, namely, a ring formation, somewhat resembling the benzene ring. It is easy to believe that such a configuration actually represents glucose better than the straight one; if it is true that the bonds of a carbon atom lie directed to the inner angles of a tetrahedron,[1] then we find that the angles subtended between these affinities are 109° 24′. A model of glucose built on this basis will become something like this:

[1] Page 178.

In a plane figure we have

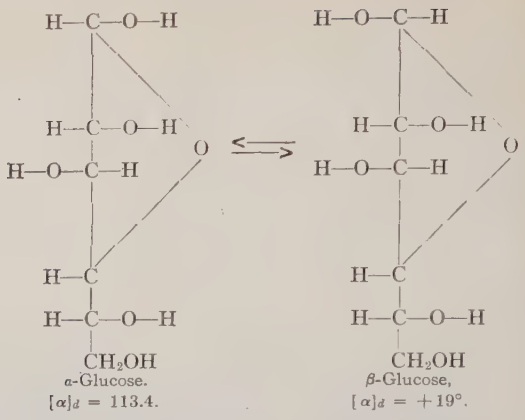


The tendency to form a ring is evident. Such a formula, however, does not answer all requirements. For example, it does not account for mutarotation,[1] which is that phenomenon seen when glucose is made into aqueous solution. The glucose becomes hydrated with the water of the solution and, in addition, it exhibits changes in rotatory power. At first the rotatory power is 110°, sinking gradually to 52.5°. This is known as α-glucose. Then it was discovered that another form of glucose arose that started with 19°, passing to 52.5°; this was termed β-glucose.[2] These are isomeres. The optical rotatory changes are supposed to be connected with the factor water, and the following formulæ are designed to represent the various factors:



[1] Latin *muta*, change.

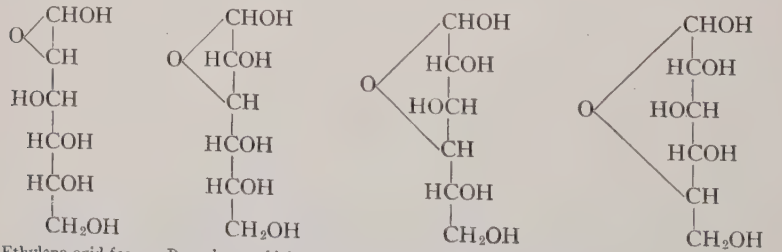
[2] These figures are those derived by Hudson, C. S., Jour. Amer. Chem. Soc., vol. 32, p. 889, 1910. The two forms of glucose may be obtained in crystalline form according to the manner of crystallization. It does not matter which form is dissolved, equilibrium is established at 52.5°.



Such a formula is that of a gamma-lactone, so called because it concerns the gamma, or third carbon below the terminal aldehyde-bearing one.[1] Such butylene rings are commonly found in hydroxy-compounds like glucose.

Summarizing, we find that the lactone formula for glucose gains the following advantages as a means of designating characteristics: (1) A formula for glucose when it is freshly dissolved in water, with a rotation of 110°. (2) A formula for glucose in solution after equilibrium has been reached, rotation 52.5°. (3) The formulæ are reversible, indicating the reversible reaction as equilibrium is being reached. (4) The aldehyde, which is the active group of the substance, is linked up into an internal anhydrid or butylene oxid formation, which makes the formula conform to the lower activity known to be possessed by glucose.

Gamma Sugars.—In the foregoing discussion it has been assumed that the ring is of the nature of a lactone, but there is no reason for eliminating other carbon atoms in the formation of the ring. Theoretically, we have the following possibilities:



[1] Page 164. The term has nothing to do with "gamma-sugar" which will be discussed presently.

The term "gamma sugar" means a reactive form. The oxygen ring is assumed at present to be in a position other than butylene, which occurs in the α and β sugars.

As Nef found, and as Levene and others have corroborated, sugar in solution presents these various configurations, but at equilibrium the larger part is in the form of either butylene or amylene. Recent work has shown that the reactive form may be an excess of the butylene configuration, and the stable form of the amylene. In such a case, the term "gamma-sugar," implying a reactive form, would be the butylene grouping.[1]

The Medical Interest in Gamma Sugars.—Blood-sugar seems to be a highly reactive sort[2] of glucid, compared with the activities of the ordinary known forms of glucose, α and β. It has been suggested[1] that the blood of the diabetic is different from that of the normal subject in having less reactive sugar, but Denis[3] and others have been unable to find any special form of glucose in diabetic blood.

Fermentation and Optical Relations.—The enzymes exhibit a specificity, choosing certain isomeres and leaving others untouched, at least at first; later, other isomeres may be attacked. The glucids mentioned above may be taken as examples of specific action, but the specificity of enzyme action has been worked exhaustively on certain compounds similar to the diglucids and known as glucosides, which will be discussed presently.[4] For the purpose here suffice it to say that there is a *d*-glucoside and an *l*-glucoside. The enzyme maltase, which splits maltose (a gluco-glucoside) into two glucose molecules readily acts upon the *d*-form, but not at all upon the *l*-form. Another enzyme, emulsin, has little or no effect upon the *d*-form, but acts readily upon the *l*-form. This enzyme specificity is found throughout biochemistry and we shall have reference to it later. It is the basis for the statement made on page 177, that glucids occurring in living things are of the *d*-series, while protids are built upon the *l*-plan.

The Polariscope (Fig. 89).—This is an instrument for the study of optical relations. Polarized light may perhaps be more readily con-

[1] Haworth, W. N. (Armstrong College, Newcastle-O-T, England). Nature, vol. 116, page 430, 1925). Hewitt, J. A. and Pryde, J. (Univ. of Wales). Biochem. Jour., vol. 14, page 395, 1920.

[2] Winter, L. B., and Smith, W. (English physiologists), Journal of Physiology (English), vol. 57, p. 100, 1923.

[3] Denis, Willey, Professor of Biochemistry, Tulane University School of Medicine, New Orleans, Louisiana. See Denis, W., and Hume, H. V., Jour. Biol. Chem., vol. 59, p. 603, 1924.

[4] Pages 188 and 189.

ceived by comparing it to sound waves which are polarized as follows (Fig. 91): Set up two synchronous tuning-forks at such a distance from one another that they beat sympathetically, that is, one wave-length or multiple, apart. When one fork is struck, the other responds.

Attach a string, loosely, between the forks as shown in Fig. 91. When the forks vibrate, the string is thrown into vibrations, represented by the "Sine Curve" undulations shown in the picture.

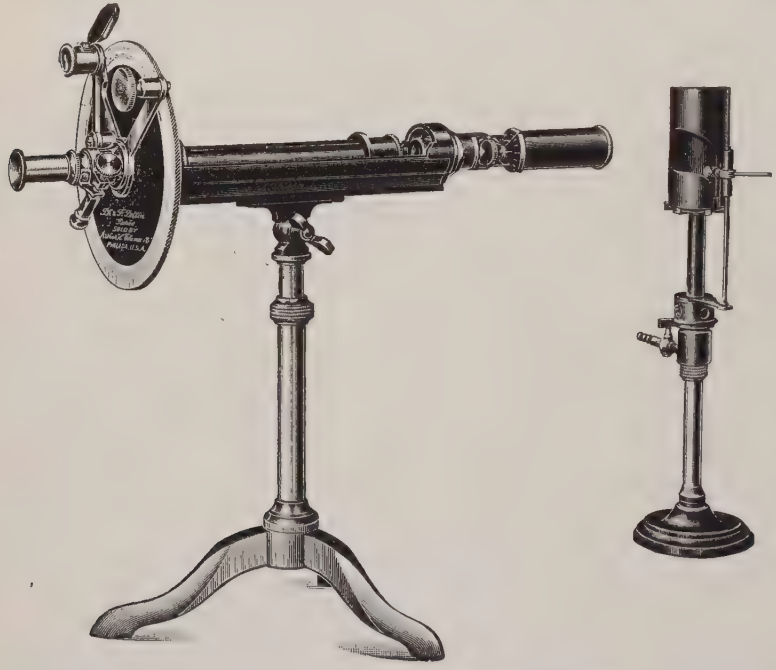


Fig. 89.—Polariscope used in biochemical analyses. The eye is applied to the ocular at the left and the object between it and the "polarizer" (extreme right); lamp, fitted for monochromatic light (sodium, or "D"-light). The "analyzer" is operated by the rotating arm on the calibrated disk to the left. A lens permits reading of the vernier.

Open the leaves of a book, vertically (P) and place them over the vibrating string. Obviously, since the string undulates in only one plane (vertical), the book will permit the waves to pass undisturbed. The string is now polarized, since only vibrations in the plane of the book pass on to the second fork. Interpose, now, a second book so that the waves may pass between the pages as in the case of the first book; the pages of both books must be in the same plane. The tuning-fork should vibrate sympathetically. Now turn the second

book so that the pages cut the plane of the waves at an angle; obviously, the waves will not pass through this book and the second fork will not vibrate sympathetically. You have crossed the planes of the book and no polarized wave can pass.

Similarly, the polariscope has two prisms, corresponding to the two books. These prisms may be composed of plates of glass, but

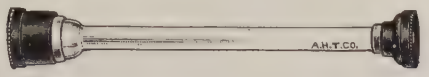


Fig. 90.—Tube used in the polariscope (Figs. 91 and 92). The tube is laid in the trough between polarizer and analyzer.

more commonly of Iceland spar ($CaCO_3$), which crystallizes in a rhombohedron, the prism being composed of many layers of these rhombs. In place of a single Iceland spar crystal Nichol[1] cut the crystal diagonally and cemented the two halves together with Canada balsam, the gum having an index of refraction less than that of the

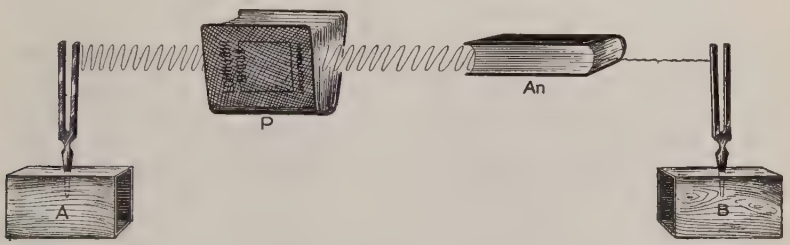


Fig. 91.—Illustration to show the principle of polarization. A wave coming from tuning-fork *A* is polarized by the leaves of the book, *P*. The waves leave *P* only in one plane. Book *An* represents the analyzer of a polariscope (Fig. 92) and as long as the plane of its leaves lies in that of *P*, the wave passes through and strikes tuning-fork *B*. If the polarizer and analyzer are "crossed," that is, if *An* is turned on its axis until its plane cuts that of *P* at an angle, no wave reaches *B*. In the polariscope the unknown, like glucose, turns the plane of polarized light (waves between *P* and *An*) and hence *An* must be turned until its plane is coincident with that of the rotated plane of light leaving the solution.[2]

spar. When light falls upon one face of the Nichol prism thus constructed, it is broken into two rays, the so-called "ordinary" and the "extraordinary." The ordinary ray, which would otherwise pass through the prism unchanged, meets the balsam layer and is totally reflected, while the extraordinary ray is transmitted as the polarized ray. If the planes of the first Nichol prism[3] and of the second

[1] Scotch physicist, 1828.

[2] For suggestions regarding this demonstration, the author thanks Professor Fredric Palmer of Haverford College.

[3] The "polarizer," producing the polarized light (Fig. 92, *P*).

prism[1] are parallel, polarized light reaches the eye. If they are "crossed," like the books above, no light passes. If, now, a substance like d-glucose solution is interposed between the parallel prisms, light will not pass to the eye, since the glucose molecules have rotated the plane of polarized light through an angle of 52.5°, which gives the same effect as if the prisms were crossed. In order to bring the light through the system again it is necessary to rotate the analyzer

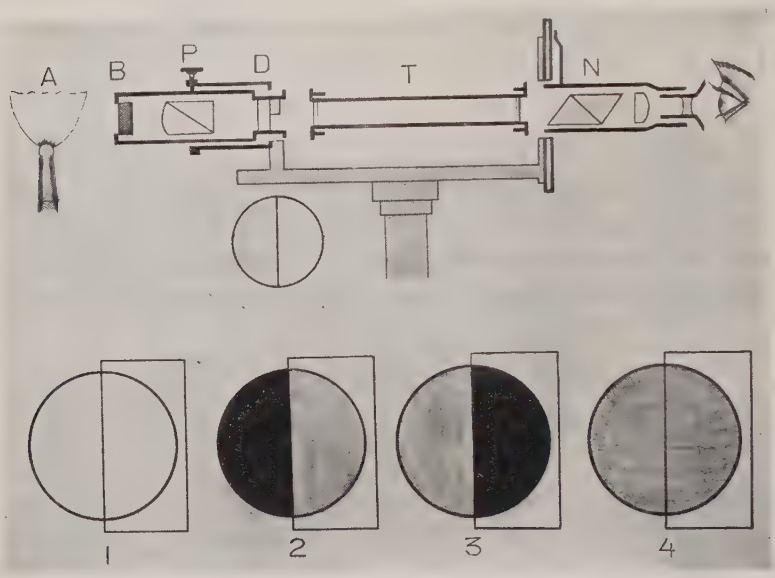


Fig. 92.—Laurent's half-shadow polarimeter: Monochromatic light (*A*) passes through the slit (*B*), through the polarizer (*P*), the diaphragm (*D*), and then through the tube (*T*) containing the unknown solution. The eye perceives the light in one of the ways shown in the diagrams 1, 2, 3, and 4, accordingly as to how the analyzer (*N*) is turned. If it is turned so that the prisms are crossed (Fig. 92), then no light enters (4); intermediate positions are in 2 and 3. If the plane of polarizer and analyzer is identical, then the eye perceives 1. (From Holland's Medical Chemistry and Toxicology.)

on the polarizer. A calibration in degrees of arc, with vernier[2] is attached to the analyzer, and hence it is possible to determine the rotation produced by the substance glucose. Such a polarizer is known as a polarized light measurer, or polarimeter.

For making a determination, monochromatic light, or light of one wave-length, is used if accuracy is desired, for the angle of deviation between the ordinary and the extraordinary rays varies with different wave-lengths, and that which is generally used is 5880Å, giv-

[1] The "analyzer," nearest the eye (Fig. 92, *N*).

[2] A subdivided scale into 0.1, 0.01, etc.

ing the yellow light shed by heated sodium at the "D" line of the spectrum. Definite distance is established between the polarizer and the analyzer and a tube of certain length is used; ordinarily a 2.2 decimeter length. Specific rotatory power (α, or alpha) is defined as the rotation of 1 gm. of the substance (like glucose) per ml. of solution viewed through a column 1 dm. (10 cms.) long with D, or sodium light (hence α_D) at a standard temperature, 20° C. Specific rotation has been found by observation for many substances. Thus glucose in 10 per cent. solution gives $\alpha_D^{20} = +52.7°$; fructose, $-93°$; galactose, $+83°$; lactose, $+52.5°$; maltose, $+138°$; sucrose, $+66.5°$; starch, $+199°$. Knowing this, one may calculate the concentration of a solution according to the formula:

$$\alpha_D^{20} = \mp \frac{a \times 100}{c \times l}$$

where

$$c = \frac{a \times 100}{\alpha_D^{20} \times l}$$

in which **a** is the observed rotation; **c** the concentration, and **l** the length of the column (tube) in dms.

Racemization.—If the optically active *d*- and *l*-isomeres are mixed in equal quantities, racemic substances are produced, which are optically inactive. The term "racemization" is derived from the Latin,[1] meaning cluster, and refers to grapes; from the grape, two kinds of tartaric acid are produced, namely, *d*-tartaric and *dl*-tartaric acid, the latter being called racemic. Pasteur[2] found that molds split racemic acid into *d*-tartaric and *l*-tartaric acids. Similar actions of enzymes in general are observed and we have already referred to the fact that *d*-glucids are affected by enzymes, while *l*-glucids are not; and that *l*-amino acids of the meat series are subject to certain enzymes, while *d*-forms are not. This property is encountered in the pharmacological action of drugs, in which physiological action depends upon whether a *d*- or an *l*-isomere is used. Thus, *l*-alkaloids are more potent than *d*-forms and Cushny[3] and others have shown that certain *d*-forms of drugs are antagonistic to other drugs in *l*-form, as in the antagonism of *l*-epinephrin and *d*-pilocarpin. "Racemization" of other substances (protids) is discussed but the two cases are fundamentally different.

[1] Latin *racemus;* Greek *rach,* berry.

[2] Page 177.

[3] Cushny, A. R. (formerly Pharmacologist, University of Michigan, late of Edinburg, Scotland). See Cushny, A. R., Text-book of Pharmacology and Therapeutics, Philadelphia, Lea & Febiger, 1924, 8th ed.

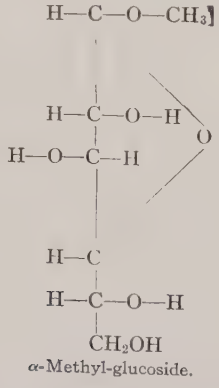
EXERCISE 21.—Under the direction of an instructor determine with the polarimeter the specific rotation of preparations[1] of glucose, fructose, galactose, maltose, sucrose, and of cornstarch.

EXERCISE 22.—In preparations made for you, determine the concentrations of the sugars, using the data given on page 187.

THE CHEMISTRY OF THE DIGLUCIDS[2]

In an earlier paragraph[3] we have discussed, briefly, the composition of the polyglucid starch, and the statement was made that it is composed of molecules of glucose. Turning to the diglucids, we find that they also are composed of molecules of the simple sugars, monoglucids, but unlike starch, there is a qualitative difference in the component sugars. Starch has but one type of sugar (glucose), while, with the exception of maltose, the diglucids are composed of two different sugars. Thus, table-sugar, sucrose,[4] is composed of glucose and fructose; lactose, of glucose and galactose.

What is the chemical linkage of the component sugars in the diglucids? It is a glucoside bond. In the case of sucrose it is a glucoside of *d*-levulose; in maltose it is a glucoside of *d*-glucose, and in lactose it is a glucoside bonding with galactose. The structural formula of a typical glucoside resembles that of glucose with a side-chain. A simple glucoside is α-methyl-glucoside:



α-Methyl-glucoside.

In α-methyl-glucoside the side-chain is the methyl radicle, CH_3. It is possible to attach a methyl group to other carbon atoms than the first. Irvine (page 177) has utilized this principle in discovering

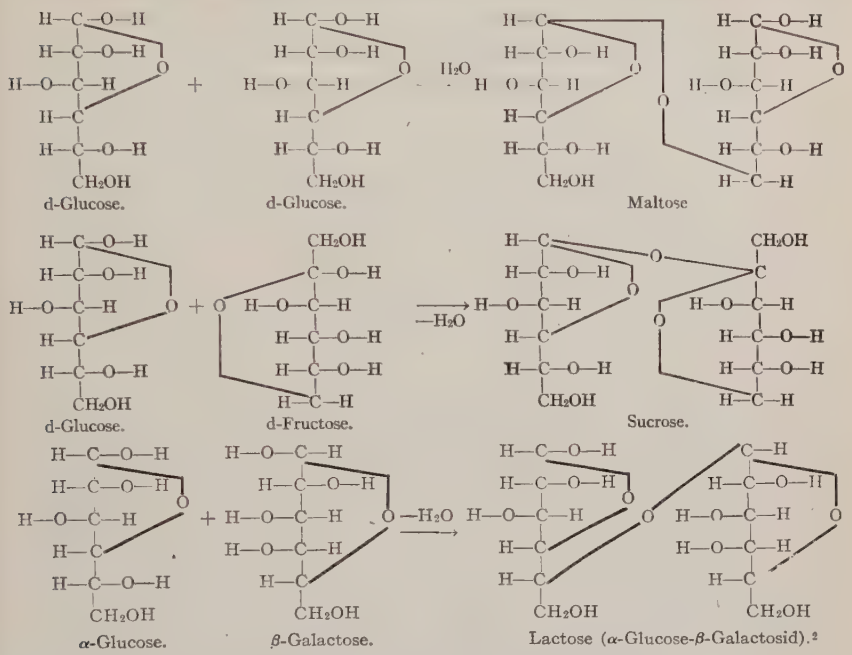
[1] These solutions have been placed in tubes, ready for use in the polarimeter.

[2] Called also disaccharids.

[3] Page 147.

[4] Cane-sugar, beet-sugar, saccharose, table-sugar, etc., being synonyms.

the structure of glucids; for by introducing methyl radicles into the hydroxides, it is possible to identify the radicle through which side-chains are attached. The following structural formulæ give the linkage in the three common diglucids, maltose, sucrose, and lactose:[1]

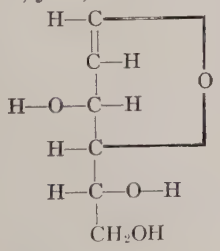


PROPERTIES OF THE DIGLUCIDS

The formulæ just given indicate the chief chemical features of the sugars. In maltose and lactose there are free terminal carbonyls, the aldehyde radicles of the first portion of the molecule; but in the case of sucrose there are no free carbonyls, carbinols being everywhere terminal. The inference is that maltose and lactose reduce copper and other reagents and sucrose does not, or, if so, only after the terminal carbinol is converted into some more active radicle. The following exercises demonstrate these facts:

[1] See Irvine, J. C., Chemical Reviews, vol. 1, page 41, 1924.

[2] Glucal, an unsaturated reduction product of glucose is of interest and should be included here.



Exercise 23.—Set up four tubes as follows: In A place 5 mls. of glucose solution; in B place a similar volume of maltose solution; in C, lactose, and in D, sucrose, using the solutions prepared for you. Add to each tube 2 drops of concentrated sulphuric acid. Leave the tubes in boiling water for two, four, six, eight, and ten minutes, at the end of each period adding 8 drops from each tube to clean test-tubes, one for each tube, A, B, C, and D. To each of the four tubes add 5 mls. of 10 per cent.[1] NaOH solution and 5 mls. of Benedict's qualitative solution. Place the four tubes in the boiling water-bath for exactly two minutes after vigorous boiling has begun and compare the colors, which indicate the relative readiness with which the different glucids become oxidized.

Exercise 24.—Make corresponding tests[2] for the times of appearance of osazones, using the same solutions as in the foregoing exercise.

Exercise 25.—*Mucic Acid Test for Lactose or Galactose.*—Principle: Galactose[3] is oxidized to its diatomic acid, mucic acid.[4] Procedure: Using the lactose solution from the previous exercises, place 25 mls. in a small Erlènmeyer flask and add 10 mls. of concentrated nitric acid. Boil intermittently (five-minute intervals) for half an hour. Restore the original volume by adding cold water, and, after thorough mixing, let stand until the next period. Filter, saving the residue on the paper. Recrystallize by adding dilute ammonium hydroxid solution to the crystals and then a few drops of concentrated HNO_3. Examine under the microscope for small, powder-like crystals.

CHEMISTRY OF THE POLYGLUCIDS

Glycogen.—Exercise 26.—Having selected a rabbit which has been well fed for a few days previous to the experiment, arrange for the excision of the liver and the immediate destruction of the enzymes capable of hydrolyzing glycogen to glucose as follows: By stomach-tube[5] administer to the rabbit a lethal dose[6] of urethane about thirty

[1] If this amount of alkali does not neutralize the acid of the solution of sugar, add more before applying the Benedict test.

[2] The method for osazones is given on page 151.

[3] Lactose is hydrolyzed to galactose in a preliminary series of reactions by the HNO_3.

[4] Page 157.

[5] A male catheter serves for this purpose. Provide a small mouth-piece, preferably made from a piece of cigar-box and whittled to the form ⌓; a hole large enough for the passage of the catheter is reamed through the center of the wooden mouth-piece.

[6] Appendix.

minutes before the operation as an anesthetic Have ready a large beaker of boiling 30 per cent. NaOH, leaving room enough to accommodate the excised liver. When ready apply an ether cone to the rabbit's nose and administer ether until the eye reflex is lost.[1] Then make a rapid incision along the ventral midline by means of a sharp scalpel from the ensiform, caudad[2] for about 5 cms. Then, with bone-scissors, cut through the sternum, cephalad,[3] exposing the liver. Place a pair of hemostats so as to isolate the biliary cyst (gall-bladder), and with scalpel, excise that organ. Lay a pair of hemostats so as to cut off all blood-supply to the liver and rapidly excise the liver, mince it, then throw it into boiling 30 per cent. NaOH, so that all parts may come into contact with the boiling lye. Boil 30 minutes. Dilute with 3 volumes of water. Filter. Allow the solution to cool; then precipitate the glycogen by adding 1.5 volumes of 95 per cent. ethanol. Dissolve the glycogen in water and filter.

Exercise 27.—*Alternate Method for Obtaining Glycogen.*—If fresh oysters or scallops are obtainable these may be used as a source of glycogen: Place four of the adductor muscles of the scallop,[4] or six oysters, in a mortar containing washed sea-sand and grind them to a mash. Precipitate the glycogen as before with ethanol, and use the filtrate for the following exercises:

Exercise 28.—*Iodin Test for Glycogen; Distinction from Starch.*—To 2 mls. of the filtrate in a test-tube add 1 drop of glacial acetic acid and 2 drops of iodin solution.[5] Compare the color developed with that of a starch solution similarly treated. Note that the glycogen gives a reddish color, while starch gives a pure blue.

Exercise 29.—*Acid Hydrolysis of Glycogen.*—To 2 mls. of the filtrate in a test-tube add 1 drop of concentrated sulphuric acid; leave in the boiling water-bath for ten minutes, neutralize with 50 per cent. NaOH solution, and test for reducing properties with Benedict's qualitative reagent.

Exercise 30.—*Hydrolysis by Means of Enzymes.*—(*a*) Saliva: Chew paraffin and collect the saliva on a filter-paper in a funnel, draining into a small beaker. Add to 5 mls. of the glycogen suspension from Exercise 26 or 27, above, 2 mls. of the filtered saliva. Leave at not over 50° C. for one minute and test the solution for the time of

[1] By touching the cornea gently it can be determined whether the reflex is lost.

[2] Toward the tail; the terms "up" and "down" are confusing in the lower mammals.

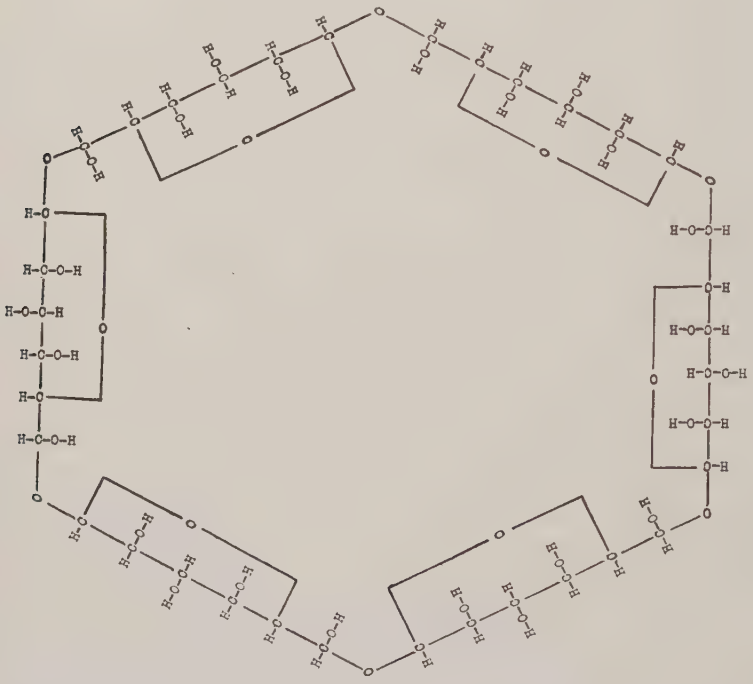
[3] Toward the head.

[4] The scallop (Pecten tenuicostata) is a large shelled mollusk living off the coast of North America. Only the large muscle that closes the shell is sold as "scallop."

[5] Page 148.

disappearance of the iodin reaction by removing a drop and bringing it into contact with a drop of iodin solution in the depression of a spot-plate. Continue heating the glycogen-saliva mixture until the color imparted by iodin disappears. Then apply Benedict's qualitative test for the presence of a reducing substance.

(*b*) Pancreatic amylase:[1] Conduct, simultaneously with the previous exercise, one involving hydrolysis of glycogen by means of the commercial "pancreatin"[2] or, better, pancreatic tissue.



Structure of starch molecule (amylose). After the specifications of Sir John Irvine.

An alkaline reaction must be present in both (*a*) and (*b*).

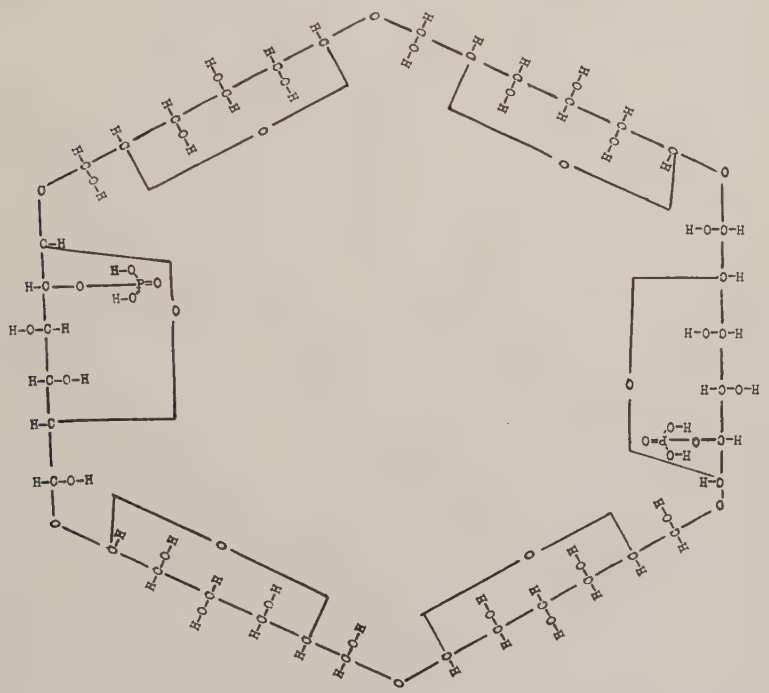
The Structure of Starch.— The structure as given in following paragraphs is that which is accepted at the present time by the majority of workers in the field. The experience of the past few years, however, teaches us to expect that this structure may undergo further changes before reaching its final form. Starch appears to be built up of units of amylose and of amylopectin (Fig. 81, page 150). The *amylose* unit is a cyclic arrangement of six 1,5-anhydro-glucose molecules (Fig. 88, is a 2.5 anhydroglucose) united in an α-glucosidal

[1] Commonly called amylopsin; see page 458.

[2] A powder containing pancreatic extract.

arrangement, the bond being to the 6-carbon atom of the adjacent molecule. During digestion, that is hydrolysis by enzymes like salivary amylase, this unit of amylose splits off glucose molecules in pairs, to give three molecules of maltose.

Amylopectin.—This unit is a cyclic arrangement of six 1,5-anhydro-glucose molecules containing two α-glucose and four β-glucose molecules, arranged as in the accompanying diagram. It is believed that the unit contains two phosphoric esters joined to the carbon atoms

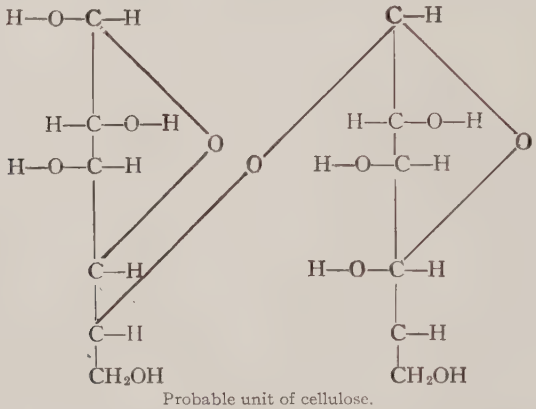


Structure of starch (amylopectin). After the specification of Sir John Irvine.

adjacent to the β-linkages on opposite sides of the ring. As in the case of amylose the bond is between the 6-carbon of one molecule and the 1-carbon of the adjacentone. During hydrolysis, the unit splits into two molecules of β-glucosido-maltose, which can be broken down into either one molecule of isomaltose and one of glucose or one of maltose and one of glucose.

Cellulose.—At the present time, it is not possible to deal with cellulose as thoroughly as we have done with starch. From analogy, it is probable that the composition of cellulose resembles, or is identical with that of amylopectin. From the investigations of Irvine

there seems little doubt that only glucose is left after complete hydrolysis. Irvine believes that an essential difference exists between the manner of association of the hexose molecules in starch and in cellulose; in starch, the linkage is between the sixth carbon atom of one glucose molecule and the first of the adjacent one whereas in cellulose, the bond is between carbon 5 and the first carbon of the next molecule of glucose. These differences are represented thus:



Probable unit of cellulose.

Cellulose is not digested by man. Through bacterial action, however, it is made available and some at least of the products are absorbed from the lower bowel into the blood. Whether "autolysis" or digestion in our colon by enzymes contained in foods consisting of cellulose contributes to our utilization of certain amounts of cellulose is not known, but such a process has been found in cattle.

The commercial value of cellulose is enormous. Many garments are made from it. Pharmacists cover their bottle-stoppers with it. When nitrated, it is used for purposes as different as ladies toilet articles and high explosives.

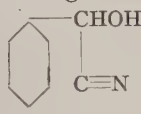
EXERCISE 31.—*Cellulose Prepared from Cotton-wool.*[1]—Using about 0.5 gram of cotton-wool, in a test-tube, add 2 mls. of diluted (1 per cent.) sodium hydroxid solution and wash with water to remove pectins. Now add bromin water,[2] and then a strong solution of sodium sulphate; wash the threads thoroughly with it. Decant and wash with water. Dry in the open air. The residue is pure

[1] Cotton-wool contains pectin as well as cellulose. For pectin see next Exercise. Cotton-wool is largely used in bacteriology in stopping tubes.

[2] If the experiment is performed under a hood, pour, very carefully, one drop of the heavy bromin liquid into the test-tube. In case of overbreathing of bromin fumes or of contact with bromin, see the Appendix for the method of treatment by anilin.

cellulose. Note the color. Note that it absorbs water from the environment.[1] Test the solubility in water, both cold and boiling; in ethanol, in weak acids, in weak bases, and in strong bases. Test the solubility in Schweitzer's reagent.[2] Add acid and obtain a precipitate of cellulose. Test the solubility in a solution of one part zinc chlorid and two of concentrated hydrochloric acid. Nitrocellulose is formed when cotton is treated with HNO_3 and H_2SO_4; 1:3 guncotton is formed. Cotton may be hydrolyzed to glucose by boiling with concentrated H_2SO_4, as in the case of starch,[3] glycogen, etc.

EXERCISE 32.—*Pectin.*—Using the commercial fluid pectin, test the solubility in water and in ethanol. Cause some to become hydrolyzed to pentose by acid; neutralize and apply Benedict's qualitative test.[4]

EXERCISE 33.—*Glucosides.*—Cherry bark contains the glucoside amygdalin. Boil the bark with a small amount of water and filter. Test the filtrate with Benedict's qualitative reagent. Add 0.5 ml. of concentrated H_2SO_4 to 5 mls. of the filtrate and boil to hydrolyze the glucoside. To a portion of the filtrate thus treated add alkali and treat with Benedict's reagent again. Reducing sugar is identified; it is glucose, which bears as a side-chain a nitrile of mandelic acid, 

. The nitrile may be detected by its conversion during the heating with acid into HCN, and the cyanid is identified by its precipitation with silver nitrate solution.

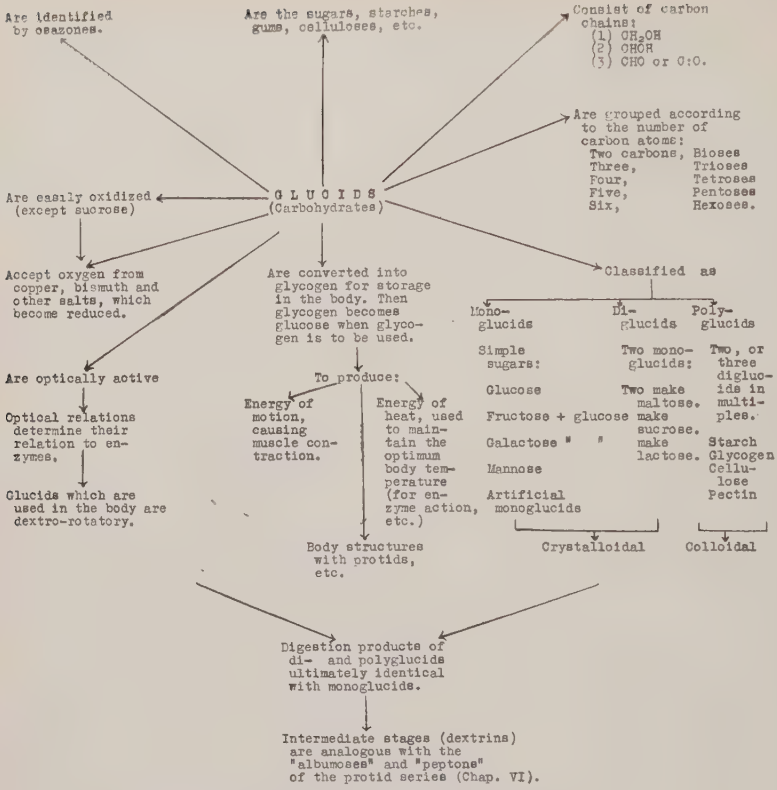
[1] It is hygroscopic. Greek *hugros*, wet, and *skopos*, watcher; that is, sensitive.

[2] Appendix.

[3] Page 149.

[4] Page 160.

GRAPHIC SUMMARY


Are identified by osazones.
Are the sugars, starches, gums, celluloses, etc.
Consist of carbon chains:
(1) CH_2OH
(2) CHOH
(3) CHO or C:O.
Are grouped according to the number of carbon atoms:
Two carbons, Bioses
Three, Trioses
Four, Tetroses
Five, Pentoses
Six, Hexoses.
GLUCIDS
(Carbohydrates)
Are easily oxidized (except sucrose)
Accept oxygen from copper, bismuth and other salts, which become reduced.
Are optically active
Optical relations determine their relation to enzymes.
Glucids which are used in the body are dextro-rotatory.
Are converted into glycogen for storage in the body. Then glycogen becomes glucose when glycogen is to be used.
To produce:
Energy of motion, causing muscle contraction.
Energy of heat, used to maintain the optimum body temperature (for enzyme action, etc.)
Body structures with protids, etc.
Classified as
Mono-glucids
Di-glucids
Poly-glucids
Simple sugars:
Glucose
Fructose + glucose
Galactose " "
Mannose
Artificial monoglucids
Two mono-glucids:
Two make maltose.
make sucrose.
make lactose.
Two, or three diglucids in multiples.
Starch
Glycogen
Cellulose
Pectin
Crystalloidal
Colloidal
Digestion products of di- and polyglucids ultimately identical with monoglucids.
Intermediate stages (dextrins) are analogous with the "albumoses" and "peptons" of the protid series (Chap. VI).

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CHAPTER V

ENERGY PRODUCERS: LIPIDS

"The principles of science have been worked out by slow degrees and much blundering by fallible human beings."—*Slosson.*[1]

Function.—The lipids, or fats and their allies, are par excellence heat producers. We have shown[2] in Chapter IV how part of the glucose molecule is burned, but the amount of heat generated in this way is only about one-half the amount of heat produced by burning an equivalent mass of lipid.

One gram of glucid on being oxidized produces 4.1 Calories.[3]
One gram of lipid on being oxidized produces 9.3 Calories.

The reason for this great heat production by the fats is that there is little oxygen and much carbon and hydrogen in the fat molecule. Moreover, the intramolecular oxygen does not give rise to heat when it unites with carbon or hydrogen during oxidation. Oxygen must be introduced from the outside to produce heat energy. The amount of oxygen necessary to burn the carbon in a glucid to CO_2 is 6 atoms per molecule of glucid. Taking glucose for an example.

$$C_6H_{12}O_6 \longrightarrow 6C \text{ and } 6H_2O.$$

Since the 6 atoms of oxygen contained in the glucose molecule are used in burning the hydrogen to H_2O, the 6 atoms of carbon must be burned by oxygen introduced from without, that is, 6 oxygen molecules.

For palmitid,[4] a common fat, the process is as follows:

Formula	$C_{51}H_{94}O_6$
Subtracting intramolecular water	$H_{12}O_6$
	$C_{51}H_{82}$ left for oxidation by oxygen from without.

[1] Slosson, E. E., chemist; head of the Science Service, Washington, D. C.

[2] Page 145.

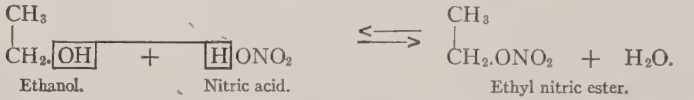
[3] A calorie (Latin *calor*, heat) is the heat necessary to raise the temperature (degree of heat referred to a standard, like that of melting ice, the boiling-point, etc.) of 1 g. substance 1° C. A Calorie (large Calorie) is 1000 times that amount of heat. The figures given above are large Calories.

[4] Following the nomenclature of the International Congress, the suffix *-id* will be used for fats.

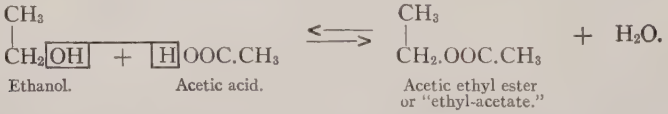
This requires 143 oxygen atoms. Contrast this amount of oxygen with that required for the burning of a molecule of glucose, given above.

Types of Lipids.—Lipids comprise the true fats, such as suet; oils, such as olive oil; the waxes, such as spermaceti; phosphorized fats, such as the so called lecithin of brain, and various special fats which are not popularly known and bear no common name. Associated with the lipids, but of no chemical relationship to them, are the sterols, such as the cholesterol found in gall-stones, and the lanolin in lambs' wool fat, used for pharmaceutical preparations.

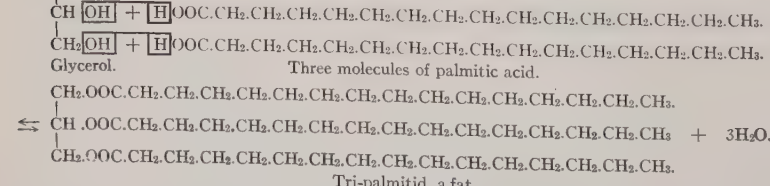
Chemical Composition.—Fats are esters of higher alcohols and fatty acids. The alcohol of the common fats and oils is glycerol.[1] In some fats the place of glycerol is taken by other alcohols or even by a glucid, like galactose. An ester is an organic "salt" of an alcohol and acid:



This reaction occurs because of the affinity of hydrogen for the hydroxyl. We have shown[2] that water ionizes to a very slight extent, which means that the ions, H^+ and OH^-, are separated only with difficulty from the molecule of water. The reaction is reversible. Using an organic acid, we have:



In these cases we are dealing with an alcohol with only one hydroxyl, but in glycerol there are three hydroxyls, each of which unites with an acid molecule; consequently, three acid molecules are used in the making of the glycerids:



All the so-called "neutral" fats, or natural fats, consist of an ester of alcohol (glycerol) and three fatty acids; hence they are called tri-compounds (tri-palmitid, tri-stearid, tri-oleid).

[1] "Glycerine," but glycerol is proper, since *-ol* indicates an alcohol.

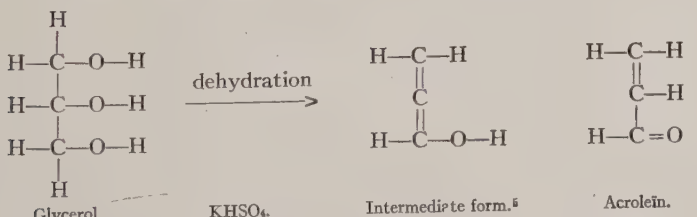
[2] Page 38.

Exercise 1.—*Detection of Fat.*— Drop upon a piece of paper a bit of the melted substance suspected of being a fat or containing fat; a grease spot is formed, which renders the paper translucent,[1] or semi-transparent. Paraffin will do much the same; apply, to another part of the paper, a drop of albolene. Note the characteristic difference between the two spots; the oil spreads and the outer margin of the spot is clear, while that of the paraffin oil is less distinct. Hold the spots over a low flame and note the spread of the fat and oil; the fat spreads more widely than the oil.

Exercise 2.—Using the spots made in the preceding exercise, hold them over a bottle of 1 per cent. osmium tetroxid ("osmic acid"); note the blackening[2] of the lipid, while the albolene remains clear.

Exercise 3.—Make additional spots as in Exercise 1 above. Apply to one set a solution of Scharlach R[3] and to another Sudan III. Note the difference in colors between the lipid and the paraffin.

Exercise 4.—*The Acroleïn Test for the Presence of Fats.*—Before making the test for suspected fat use pure glycerol. Heat a few drops of glycerol with some powdered potassium hydrogen sulphate in a pyrex test tube taking care not to burn the material. Note the pungent, characteristic odor of acroleïn. Now repeat the experiment, using the suspected fat; if the fat is solid, dissolve it in a small amount of carbon tetrachlorid, CCl_4.[4] Note the odorof acroleïn. Acroleïn is acrylic aldehyde removed by dehydration from glycerol:



Glycerol. Intermediate form.[5] Acroleïn.

[1] Before the introduction of glass (discovered by the Phoenicians in their camp-fires built upon the sands, quartz being fused to glass), oiled paper was used instead.

[2] If the osmic preparation is old, a drop may be applied to the spots.

[3] This dye is used in making the "scarlet ointment" of the drug-store, which is sold for the treatment of burns, the principle being that the dye kills the half dead cells surrounding the burn and leaves an impulse for the development of new cells, which close the wound.

[4] Carbon tetrachlorid is non-inflammable. It is used in fire extinguishers and in non-inflammable cleaning mixtures.

[5] The hydroxyl is united with an unsaturated carbon atom, which is unstable, and a shift takes place to satisfy the open bond to make the aldehyde group.

Since acroleïn is an aldehyde it possesses reducing properties for metal hydroxides, like silver hydroxid, which is made as follows:

$$AgNO_3 + NaOH \longrightarrow AgOH + NaNO_3.$$

Dampen a piece of filter-paper with silver hydroxid solution to which a little ammonium hydroxid has been added and hold the paper tightly over the mouth of the test-tube containing the acroleïn solution. Boil and note the deposition of metallic silver as a brownish spot on the paper. Fat may be used in place of pure glycerol, since there is an hydrolysis of the fat, freeing glycerol. Verify this statement, using a CCl_4 solution of tallow.[1]

Exercise 5.—*The Absorption of Iodin by Fats.*—Using oleic acid:

$$CH_3.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.HC=CH.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH.$$

To 2 mls. of the acid in a test-tube add 5 mls. of water and, drop by drop, 0.01 normal iodin solution. Note the disappearance of the brown color of the iodin until a certain point has been reached, after which the addition of iodin no longer is followed by a discharge of the brown color; this is the point of saturation. Repeat, using 1 drop of olive oil dissolved in 5 mls. of carbon tetrachlorid. The amount of iodin absorbed by fats is a factor in their identification. The following Exercise gives one of the methods:

Exercise 6.—*Method of Wijs.*[2]—Principle: A known amount of fat is subjected to an excess of iodin. Iodin is readily absorbed by the fat. The amount of iodin remaining after exposure to fat can be titrated with sodium thiosulphate, thus making possible the calculation of the iodin value, that is, the index of the iodin-absorbing power of the fat. Procedure: Weigh, accurately, about 0.3. g. olive oil into a 250-ml. Erlenmeyer flask and dissolve the oil in 10 mls. chloroform. Now add exactly 25 mls. of Wijs'[3] solution by means of a 25-ml. pipette. Stopper the flask and leave in a dark place for half an hour. Then add 15 mls. 10 per cent. potassium iodid solution and 100 mls. H_2O. Titrate the solution against decinormal thiosulphate solution. This is done by running in the thiosulphate until the above solution becomes straw color, after which 5 drops of starch solution[4] are added. Now titrate again until the iodin is decolorized. Standardi-

[1] The tallow should be "rendered" by melting it, letting it stand, and skimming off the fat from the protid.

[2] Wijs, H., chemist of the Netherlands; the word is pronounced "vayze."

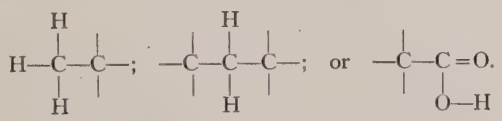
[3] Appendix.

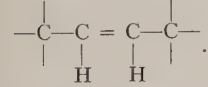
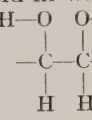
[4] Page 147; see Appendix for Lintner's Soluble Starch.

zation of the thiosulphate in terms of iodin: Repeat the former titration, using in place of the oil the iodin solution itself to which 15 mls. 10 per cent. KI are added. The difference between the titration figure for the oil and that for the iodin represents the amount of iodin absorbed by the oil. Express as per cent. The student may be aided by an arbitrary example:

Titration figure for the unknown after standing in the dark 35 mls. 0.1 n. "hypo."
Twenty-five mls. of the Wijs' solution alone titrate..... 60 " " " "
Therefore, the amount iodin absorbed is 60 − 35 = ... 25 " " " "
Atomic weight iodin = 126.92; an 0.1 normal solution is... 0.012692 g. per ml.
Then, 25 mls. 0.1 normal thiosulphate = ... 3.173 gs. iodin.
Converting to per cent., $\frac{100}{\text{Figure in grams of oil weighed}} \times 3.173 = 105.978$, the iodin number of the oil.

Nature of Iodin Absorption.— Not all fats and oils absorb iodin. Thus, if members of the acetic acid series, like palmitid or stearid, which are found in tallow, are treated with iodin, or other halogen, like bromin, no absorption occurs.[1] An examination of the structural formulæ of these fats, like that of tripalmitid,[2] shows no provision for iodin absorption, for all the valencies are filled, each carbon atom having its bonds satisfied in one of the following ways:



Oleid,[3] however, belongs to a different class, being one of the series to which acroleïn belongs.[4] A glance at the formula shows that two carbon atoms in the middle of the chain have double bonds, thus:  . One molecule (two atoms) of iodin are absorbed by one molecule of oleic acid and three for the oil, trioleid. The reason for placing the unsaturated carbon atoms in the middle of the chain is that when oleic acid is oxidized there is first formed an acid in which these unsaturated bonds are satisfied with hydroxyls:  .

[1] If the student try to verify this, he may find a slight absorption, due to the factors that were described on page 114, that is, adsorption. This phenomenon is quite different from iodin absorption, as we have described it above.

[2] Page 198.

[3] See the formula for oleic acid, Appendix, "Characteristic Biochemical Configurations."

[4] Compare the structural formula for acroleïn, page 199.

This acid is known as di-hydroxy-stearic acid; it forms by cleavage of the molecule between the carbons bearing the hydroxyls, two acids, each representing one-half of the original oleic acid molecule, one acid becoming pelargonic acid:

$$CH_3.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH.$$

and the second acid becoming the dicarboxylic acid, azelaic acid:

$$HOOC.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH.$$

The unsaturated carbon atom, then, represents the place of rupture of the molecule. Oxidation at the double bond causes color.[1]

Hydrogenation of Fats and Oils.—In place of halogens, or hydroxyls, hydrogen atoms may be used to satisfy the unsaturated carbon atoms and are so used in the arts. We have just seen that by saturating oleic acid with hydroxyls, we obtain iso-oleic or elaidic acid, or by hydrogenation stearic acid which belongs to the saturated, or acetic acid series. The members of this series occurring in common fats and oils have higher melting-points than those of the acrylic series:

Tri-oleid	melts at −5° C.
Tri-palmitid	at +62.0
Tri-stearid	at +71.6

If, by introducing hydrogen into the molecule of the fats and oils which are liquid at ordinary temperatures, they can be converted into fats which are solid at these temperatures, packing of the products is facilitated and there is also less danger of the substances spoiling during commercial handling. This process is extensively used in making butter substitutes.

Oxidation of Fatty Acids with More than One Bond.—Linolic (linoleïc) acid:

$$CH_3.CH_2.CH_2.CH_2.CH_2.\overset{H}{\underset{|}{\overset{|}{C}}}=\overset{H}{\underset{|}{\overset{|}{C}}}.CH_2.\overset{H}{\underset{|}{\overset{|}{C}}}=\overset{H}{\underset{|}{\overset{|}{C}}}.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH.$$

occurs as a triglycerid accompanying olive oil. It has been obtained also from hydrolysis products of lecithin,[2] which undergoes oxidation readily probably because it contains unsaturated fatty acids of the series we are considering. White lecithin (unoxidized) is rare.

Another acid is used as a "drying oil" in painting; It is linolenic

$$CH_3.CH_2.\overset{H}{\underset{|}{\overset{|}{C}}}=\overset{H}{\underset{|}{\overset{|}{C}}}.CH_2.\overset{H}{\underset{|}{\overset{|}{C}}}=\overset{H}{\underset{|}{\overset{|}{C}}}.CH_2.\overset{H}{\underset{|}{\overset{|}{C}}}=\overset{H}{\underset{|}{\overset{|}{C}}}.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH.$$

[1] Pure oleic acid, free from oxidation, is colorless.

[2] Page 210.

acid: It is found in linseed oil. It readily gives up oxygen to the wood upon which the oil is laid, the wood in its turn becoming oxidized and hence preserved. The unpleasant odors from dead fish are due to the oxidation of unsaturated oils causing rancidity which is partly a process of oxidation, partly hydrolysis, and partly the action of bacteria and of moulds. The exact nature of rancidity is obscure.[1]

Castor oil is largely (90 per cent.) an unsaturated hydroxy-acid, ricinoleic acid:

$$CH_3.CH_2.CH_2.CH_2.CH_2.CH_2.CH(OH).CH_2.HC = CH.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH.$$

The presence of hydroxyls causes ricinoleic acid to show some of the characteristics of alcohols (solubility in alcohols, ester formation with acids, etc.). When ricinoleic acid is saturated it becomes ricinelaidic acid.[2] Another acid, saturated, bearing two hydroxyls, also occurs in castor oil; it is known as di-hydroxy-stearic acid (1 per cent.) referred to on page 202.

The Physiological Interest in Unsaturation.—From the work of Leathes[3] it is evident that unsaturation is a factor in the metabolism of the fats, for one may distinguish between "depot" fat and organ fat. Depot fat is deposited in the great fat storage places of the body—over the kidneys, in the great omentum, etc. Organ fat lies in the tissues themselves, like the liver. Cod-liver oil has been known for years as a readily assimilable oil, quite distinct in nutritional properties from suet and other depot fats. Leathes has shown that in fat metabolism the saturated fats are mobilized from the fat depots and carried by the blood-stream from the region of the kidney and elsewhere to the liver, where the process of desaturation is accomplished. Thus the fat is made ready for combustion.[4] Outside the body unsaturated fats burn no more readily than saturated ones, but within the body the opposite is true, in fact, it is probable that saturated fats are not burned as such in the organism.[5] There must, therefore, be some chemical change which accompanies desaturation, rendering the fats available for oxidation. Exactly what this change

[1] For an account of rancidity see Kerr, R. H. and Sorber, I. G. Industrial and Eng. Chem., vol. 15, page 382, 1923.

[2] Page 202.

[3] Leathes, J. B., English biochemist, Sheffield University. See Leathes, J. B., The Fats, New York, Longmans, Green & Co., 1910.

[4] The powder is dried, as has been remarked.

[5] Bloor and his pupils recently have obtained evidence for the oxidation of saturated fats.

is remains unknown, but we have seen how oleic acid becomes divided at the point of unsaturation, each half of the original oleic acid being converted into an independent acid. Each of these acids then loses one carbon atom by the process of decarboxylation[1] and then is burned to CO_2 and H_2O by the process known as beta-oxidation.[2] Whatever the reason for this desaturation procedure, the tissue fats of the body have properties not possessed by fats of the acetic acid series.

The Character of Body Fats.—The characteristics of body fat depend upon several factors, such as their location in the body, and the type of food from which they originate. In man the content of unsaturated fats in various parts of the body as measured by the iodin number is as follows (Abderhalden, after different authors):

Source.	Iodin number.
Subcutaneous fat, abdomen	66.3
breast	64.4
Kidney, depot fat	57.8

Human fat consists of three fats, proportionated according to their fatty acids as follows:

	Per cent. of total fatty acids hydrolyzed.
Stearic acid	4.9– 6.3
Palmitic acid	16.9–21.1
Oleic acid	65.6–86.7

It is probable that the first and third acids exist in the fat as a dioleo-stearid compound, tripalmitid, accompanying this compound in simple physical mixture. The subcutaneous fat of the human infant is more saturated than in the adult. The fat is least liquid at birth (48° C.) and becomes of lower melting-point as age progresses (37° C. at 17 mos.). There is a larger proportion a birth of tripalmitid than later. Human fat may melt at as low a temperature as 17.6° C. as contrasted with the average melting point of lard (hog-fat), 27.6° C. and tallow (mixed ox- or sheep-fat), 45° C. Inasmuch as these body fats are nearly neutral,[3] they are commonly called "neutral fats."

Butter Fat.—This is a tissue fat and hence we should expect it to have a low melting-point. In fact, it is an oil. The limits of melting

[1] Page 253.
[2] Page 531.
[3] They are neutral to litmus.

of the average butter fat derived from cow's milk are between 28° and 35° C. The iodin number varies between the same numbers (25–35). Unlike the depot fats, butter fat contains volatile fatty acids, butyric 6 per cent.:

$$CH_3.CH_2.CH_2.COOH$$

Caproic:

$$CH_3.CH_2.CH_2.CH_2.CH_2.COOH$$

Caprylic:

$$CH_3.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH$$

Capric:

$$CH_3.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH$$

these three making up 3 per cent. Myristic (see Fig. 9):

$$CH_3.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.COOH$$

associated with palmitic and stearic when quantitative determinations are made. Other acids are formic, H.COOH; acetic, CH_3COOH; lauric, $CH_3(CH_2)_{10}COOH$; and arachidic $CH_3(CH_2)_{18}$-

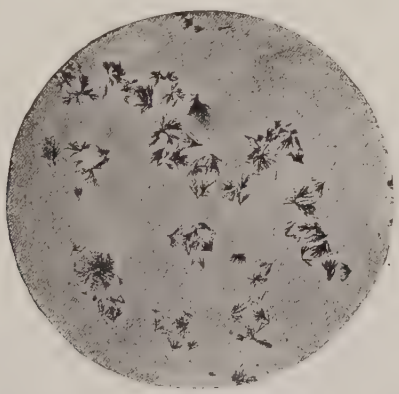


Fig. 93.—Crystals of cocoanut oil, magnified 45 times. Compare Fig. 171. (From Woodman, Food Analysis, McGraw-Hill Book Co., New York, N. Y.)

COOH. Glycerol and other substances are found in quantitative analyses. Hence, the presence of butter may readily be detected by distilling off the substances at comparatively low temperatures (or rapidly at the boiling-point of water, 100° C.) and titrating the distillate with standard alkali. This is the principle of the Reichert-Meissl and similar modifications of the original Reichert method for the estimation of volatile constituents of the fats by various chemists,

like Wollny, Polenski, Leffmann, and others. The Leffmann[1] procedure is given herewith:

The Leffmann method for quantitative determination of volatile acids: Weigh 5 gs. fat into a small Kjeldahl (300-ml.) flask. Add 20 mls. of the special glycerol solution (Appendix). Heat over a free flame[2] until the foaming ceases and the mixture becomes perfectly clear (five minutes). Add 135 mls. distilled water in small amounts to prevent foaming. Add pieces of pumice. While rotating the contents of the flask under the cold-water tap, add 8 mls. 25 per cent. sulphuric acid solution[3] and immediately attach to a Liebig condenser,[4] receiving the distillate in a 250-ml. volumetric cylinder. When 110 mls. of distillate have been collected,[5] disconnect and, after thoroughly mixing the contents of the cylinder, filter through a dry filter. Titrate exactly 100 mls. filtrate with 0.1 normal NaOH solution (phenolphthalein).

Calculation: Multiply the figure obtained by titration by 1.1. If exactly 5 gs. of fat were weighed for the determination, the Reichert-Meissl number is given directly, but if the weight is not exactly 5.0 gs., then a proportion must be made, since the factor is meant for that weight, and also for exactly 110 mls. of distillate. Example:

4.9 gs. fat were weighed.
100.0 mls. of distillate were obtained.
30.0 mls. of 0.1 normal NaOH were used in titration.
Then: $30 \times 1.1 = 33.0$ mls.[6]
For 4.9 gs. in place of 5.0, $4.9 : 33 = 5 : x$.
$x = 33.6$ Reichert-Meissl number.

If it is desired to determine the content of acetic acid, the acetyl number is determined by acetylating the fat in the distillate by means of acetic anhydrid and then titrating with standard alkali. The acetyl number is the amount of KOH by weight required to neutralize the acetic acid volatilized from 1 g. of fat after acetylating. By using the data of such determinations it is possible to discover adulteration in butter:

[1] Leffmann, H., Chemical Pathologist, Emeritus, Jefferson Hospital, Philadelphia. See Leffmann, H., and Beam, William, Analyst, vol. 16, p. 153, 1891.

[2] The heating may be done by holding the flask by means of tongs directly over the flame if resistance glass, like pyrex, is used.

[3] This sets free the acids. The sulphuric acid must be added slowly and with care.

[4] If the Kjeldahl condenser has been well washed by distilling 300 mls. of water through it, it may be used for such distillations.

[5] This should take about half an hour.

[6] 1.1 is the ratio $\frac{110}{100}$.

Reichert-Meissl number for:	
Butter	30.00
Lard	0.68
Tallow	0.50
Cocoanut oil	7.00
Olive oil	0.60

CLASSIFICATION

The following classification of lipids follows the scheme of Bloor:[1]

Lipids, substances:

(*a*) Insoluble in water; soluble in fat-solvents (ether, etc.).

(*b*) Related to fatty acids as esters, actual or potential.

(*c*) Utilized by living organisms.

I. Simple lipids. Esters of fatty acids with various (higher) alcohols:

(*a*) Fats—esters with glycerol.

(*b*) Waxes—esters with alcohols other than glycerol.

II. Compound lipids. Esters containing groups in addition to an alcohol and fatty acids.

(*a*) Phospholipids: Glycerids of fatty acids with nitrogen and phosphorus. Called "Phosphatids" by Thudicum.

(1) Lecithin, in tissues in general; soluble in alcohol; unsaturated acids are oleic and arachidonic. The nitrogenous part is cholin.

(2) Kephalin, in nervous tissue, especially; insoluble in ethanol; the unsaturated acid is kephalin-linolic acid; the nitrogenous part is amino-ethyl-alcohol.

(3) Cuorin, in heart tissue; insoluble in ethanol; the unsaturated acids are linolic and linoleic; the nitrogenous part is unknown. Cuorin may be a mixture rather than an individual compound.[2]

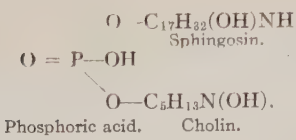
(4) Sphingomyelin, in brain, principally, but also in other tissues. Insoluble in ethanol; precipitated by aceton. Levene finds an unnamed hydroxy-acid, the unsaturated bonds satisfied with OH. Lignoceric acid also present. Since the acids are saturated, sphingomyelin does not become altered on standing. There are two nitrogenous compounds, namely, cholin and sphingosin. Tentative structural formula for sphingosin:

$$CH_3.(CH_2)_{11}.HC{:}CH.CHOH.CHOH.CH_2.NH_2.$$

[1] Bloor, W. R. Biochemistry of the Fats. Chem. Reviews, vol. 2, page 243, 1925.

[2] Cuorin undoubtedly is not a chemical entity. See Maclean H. Lecithin and Allied Substances, 2nd ed., London, Longmans Green and Co., Ltd., 1927.

The formula for sphingomyelin is given as:



(*b*) Galactolipids: Compounds of fatty acids containing no phosphorus. Instead of glycerol, galactose is present. The fatty acid is phrenosinic or lignoceric acid.[1] These substances were called, collectively, cerebrosides by Thudicum.

(1) Phrenosin: Soluble in 85 per cent. ethanol, insoluble in ethyl-ether. The nitrogenous part is sphingosin. The fatty acid is phrenosinic acid, which resembles stearic acid. Crystallizes both as liquid crystals[2] and as formed crystals. Resembles lecithin in forming myelin-forms in water. The optical relations of these substances have been studied in pyridin solutions, which are dextrorotatory.

(2) Kerasin: Precipitated from hot ethanol in groups of finely spun crystals, the groups adhering and causing the formation of a gelatinous consistency of the residue from hot ethanol. In amounts above one part to 321 of ethanol, kerasin is soluble; this property is utilized in purifying the crystals. In all other respects kerasin resembles phrenosin, save one: The acid is lignoceric acid. In pyridin solution it is levorotatory.

In an earlier section[3] it was suggested that galactose may be an indispensable constituent of food for the growing child, because it is a part of the nervous system. We are enabled to understand this reference now, for it is in the galactolipid that galactose is found to the greatest extent in the body. Galactose enters the body in milk and also in green leaves, in which it forms a part of the supporting structure of the cells (galactosans). Galactose may enter the body, also, when brains are ingested as food. Galactose is converted into glycogen and is stored as such, but there is no evidence that galactose can form from glycogen, although it may do so by way of glucose.

[1] Until the discovery of this acid in phospholipids and galactolipids it had been found only in coal-tar.

[2] These substances have been studied by Lehmann. For an account of his discoveries see Mellor (citation on page 21), vol. 1, p. 645.

[3] Page 147.

Chemistry of Lecithin.— This phosphatid occurs in all actively growing cells, in nervous tissue, and in the yolk of eggs. Its occurrence is suggestive of an important rôle, either as a by-product, a precursor, or an active metabolite of the cell. The fact that it contains in its chemical composition some of the configurations found in the characteristic compound of the cell nucleus—the vital part of the cell—leads to the assumption that lecithin may contribute to the formation of the "chromatin" of the cell, which is associated in the

Fig. 94.—Walter R. Bloor, Professor of Biochemistry, Rochester School of Medicine, Rochester, N. Y. Student of lipids, creator of methods for analyses of blood-lipids.

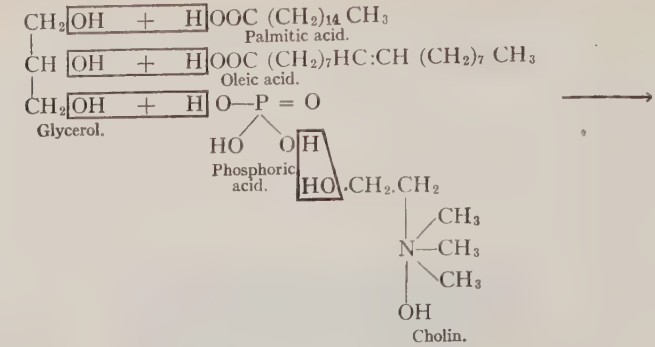
minds of biologists with hereditary transmission, sex, etc. Lecithin affords a means of transport in the blood of the unsaturated fatty acids (Bloor.)[1]

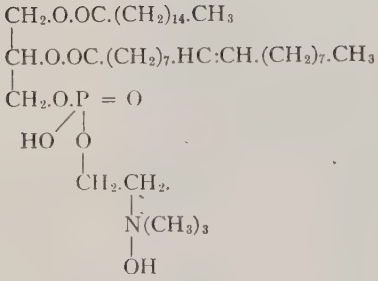
The following diagram gives the general composition of the molecule of lecithin:

GLYCEROL
1. FATTY ACID (saturated)
2. FATTY ACID (unsaturated)
3. PHOSPHORIC ACID
NITROGENOUS RADICLE
(cholin, in lecithin)

[1] Bloor, W. R., Fig. 94.

Completed formula for a molecule of the phosphatid, lecithin:





Molecule of lecithin.

The reasons for believing that all lecithins contain at least one unsaturated acid are the iodin number and the readiness with which the compound, which in its purest state is white, becomes brownish by oxidation. The iodin number varies for lecithin derived from different sources in the body:

Lecithin from:

	Iodin number.
Egg-yolk, fresh	69.0
old	48.7
Heart (beef)	100.0
Liver (beef)	63.0
Testicles (beef)	117.0
Thyroid (beef)	69.0

Besides the unsaturated acid, oleic, linolic,[1] and, recently,[2] arachidonic, another unsaturated acid of the acrylic series has been found. The presence of unsaturation in lecithin is of the greatest interest, for it leads to the belief that this compound plays an active rather than

[1] Formula, page 202.

[2] By P. A. Levene, head of the Department of Chemistry, Rockefeller Institute, New York; portrait, Fig. 95.

passive rôle in the cell, inasmuch as lecithin is labile and may possibly be linked with protid metabolism. Through the work of Levene and others it is evident that many different kinds of lecithins exist, characterized by the presence of certain kinds of unsaturated acids. Thus, Levene found four different lecithins in liver, the fatty acids consisting of:

	Saturated.	Unsaturated.
Lecithin I	Palmitic	Oleic
Lecithin II	Stearic	Oleic
Lecithin III	Palmitic	Arachidonic[1]
Lecithin IV	Stearic	Arachidonic

In egg-yolk Levene found three lecithins, combinations of palmitic and stearic saturated acids, together with the unsaturated

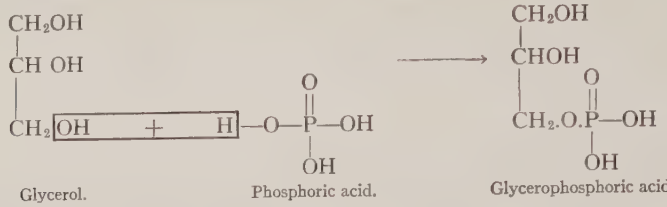
Fig. 95.—P. A. Levene, Director of the Rockefeller Institute Department of Chemistry. Intensive investigations on the pure chemistry of numerous biochemically important substances.

acids—oleic, linoleic, and arachidonic. Hence the term "lecithin" is a general one and the especial type should be designated in each case.

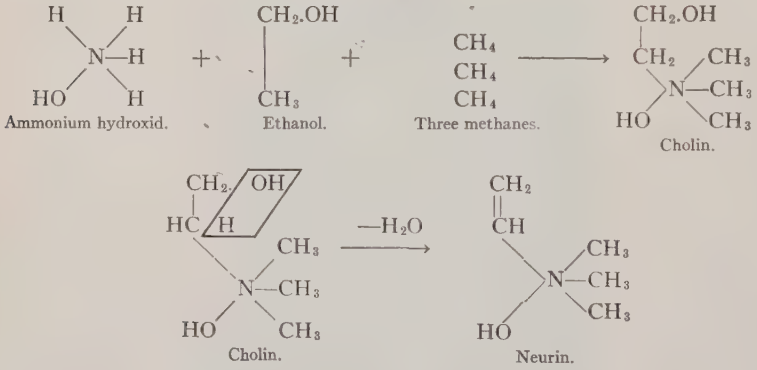
The Chemistry of the Nitrogenous Parts of the Phospholipids.—The alcohol, glycerol, or its ally, galactose, may be separated from

[1] A 20-carbon, unsaturated acid.

the nitrogenous portion of the molecule of a phosphatid, as a glycerophosphoric acid.[1] The composition of glycerophosphoric acid is:



In the case of cholin, which is closely related to substances of powerful pharmacological action, like neurin and muscarin, the nitrogenous portion separated off is a tri-methyl-amin. The interrelationships of these three substances are shown as substituted ammonium hydroxid:



All of these tri-methylamin compounds are highly toxic, stimulating the terminals of certain nerves, like the vagus or tenth nerve. Cholin does not ordinarily occur freely in the body, but may be absorbed after intestinal putrefaction. The ordinary digestive juices do not cleave lecithin to free cholin, but autolytic processes[2] do. Cholin occurs in certain drugs, like ergot. Cholin is an excellent example of the correlation of chemical configuration and pharmacological action. Thus, if the ethanol radicle in cholin is acetylated, a compound results which is one hundred times as potent as cholin in lowering the blood-pressure, but the toxicity is decreased only three times.

The nitrogenous part of other phosphatids, such as kephalin, consists of substances somewhat similar to those found in cholin. Keph-

[1] See New and Non-official Remedies, Chicago, Amer. Med. Assoc.; issued in revised form at frequent intervals. Glycerophosphates are used in therapeutics.

[2] Page 461.

alin contains amino-ethanol: , nitrogen being trivalent, not pentavalent as in cholin.

Exercises on the Phospholipids

Exercise 7.—*Preparation of "Lecithin" from the Brain.*—Using the brain material which has been dried in a current of warm air (40° C.), ground in a mill, and powdered in a mortar, transfer 100 gs. to a 250-ml. Erlenmeyer flask and add one volume of ethanol, 95 per cent. Shake the contents thoroughly and filter through dry paper. Repeat this procedure twice and save the filtrates. Then add one-half volume of ethanol ("absolute," 99.6 per cent.) and combine the filtrate with that from the previous filtrations. After each extraction the material may be passed through cheese-cloth previous to filtration through paper, in order to conserve time. Concentrate the combined filtrates on the water-bath at about 55° C. until a syrup of thin consistency is obtained. Dissolve the residue in a small volume of ethyl ether. Some of the material goes into solution and much remains insoluble; but without attempting further separation add aceton and mix thoroughly with a pestle. Strain through muslin and filter through paper, saving both fractions. To the residue add another portion of aceton and repeat the extraction. Combine the filtrates, transfer the residue to a mortar, and make an emulsion with a large excess of saline (4 volumes). Add one-half volume of aceton to the emulsion. Remove the floating white flaky material by means of a stirring-rod and complete the preparation by filtering through paper which has been perforated with the sharp end of a lead-pencil. This white residue contains lecithin. Dissolve in ether and centrifuge the ether from a precipitate; this is sphingomyelin. Decant the ether layer and add one volume of aceton. Centrifuge the solution and find the lecithin as the residue at the bottom of the tube. Decant the supernatant liquid. Dissolve the residue in "absolute" ethanol. If the solution is murky, it contains a suspension of kephalin. This may be centrifuged from the supernatant layer, which is saved, concentrated on the water-bath, and dried in a desiccator[1] over sulphuric acid. If the procedure has been carried out properly, the lecithin obtained is quite pure and not discolored with oxidation products which will render it brownish.

[1] Page 93.

EXERCISE 8.—Place a bit of the lecithin on a microscope slide and add a drop of water. Note the "myelin" formation—finger-like extensions somewhat like pseudopodia of Amœba. Warm the slide and note that the material becomes brownish, owing to oxidation. Transfer a small portion of the lecithin to a small test-tube and add 5 drops of 10 per cent. NaOH solution. Boil and note the "fishy" odor, due to tri-methylamin formed in the decomposition of the cholin. Test for the presence of phosphoric acid by thoroughly mixing lecithin and dry powdered KNO_3 in a crucible. Warm gradually, then more rapidly, and continue heating until the dark color has disappeared. Let the crucible cool, add water to dissolve the residue, and make the ordinary test for the presence of $-PO_4$.[1] On another sample of lecithin make the acroleïn test.

THE STEROLS

Associated with the true lipids are certain substances related to the aromatic groups, but having no chemical affinity for the fats.[2] For want of a more logical place of introduction these substances, known as *sterols*, are commonly discussed with the true fats, to which they bear only physical relationships. Some fat solvents (hot ethanol, ethyl ether, chloroform, carbon tetrachlorid, and carbon bisulphid) will dissolve the sterols, but others (cold ethanol) will not. Conversely, some solvents for cholesterol will not dissolve all lipids (aceton).

There are three principal groups of sterols:

(1) **Phytosterols.**—Crystalline form, hexagonal. Melting-point of the crystals, 137° C. The acetate melts at 125° C. Occurrence: Plants, especially seeds.

(2) **Cholesterol.**—Crystalline form, quadrilateral, with characteristic notched edges.[3] Acetate crystals melt at 114° C. Occurrence: Animal structures, like gall-stones (whence the name, Greek *chole*, bile; *stereos*, solid); in nervous tissue and in rapidly growing cells. Occurs in fairly constant concentration in normal human blood (150 mgs. per 100 mls. whole blood), increasing in pregnancy and in some diseases. Present in diabetes and in atheromatous arteries in arteriosclerosis. Soluble in bile-salts solutions and hence in bile.

[1] Page 360.

[2] However, recent investigations point to a universal association of these cyclic compounds as esters of fatty acids.

[3] The form of cholesterol crystal described is the one commonly found, but this is formed under the stress of surface tension. Since this is the characteristic form obtained except under special methods of procedure, it alone is described here.

Exists as such, or as esters in the fat of wool, hence the water-repellant action of such materials as Harris tweed, from which the lanolin has not been removed; the lanolin of the pharmacist, etc. Dissolved by fat, it serves in the latter case as a medium for dispensing mercury, etc., in ointments.[1] Separable from the esters by making a digitonin-cholesterid[2] in which the esters are insoluble. The sterols, especially an ally of cholesterol, have been associated with the curative factors in rickets.[3] It is now known what especial chemical configuration is responsible for the favorable action in the treatment of this disease.

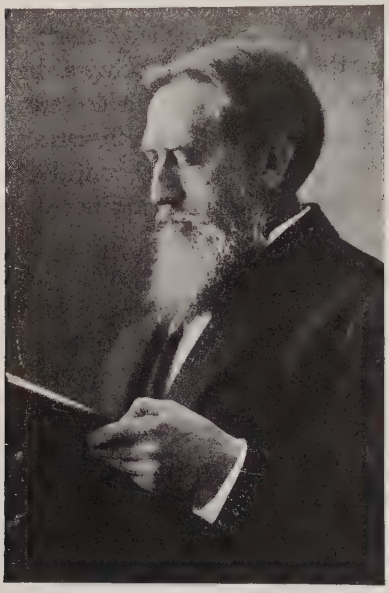
(3) **Koprosterol.**[4]—Cholesterol-like substances of the feces, probably formed from cholesterol by reduction. Melting-point of the crystals, 95° C. Derived products of phyto- and cholesterols, such as koprosterol have no curative effects.

Exercise 9.—Grind up a portion of a gall-stone in a mortar and dissolve the mass in a mixture of ethanol ("absolute") and ethyl ether. Filter. Transfer the filtrate to a small beaker and evaporate the solvent on the water-bath. The residue contains practically pure cholesterol. Transfer some of the material to a microscope slide and examine under the microscope for squarish crystals with characteristically notched edges, the whole resembling a number of microscope cover-glasses piled upon one another with some of the corners broken away.

Exercise 10.—*Tests for the Presence of Cholesterol.*—Salkowski's test:[5] Dissolve a small amount of cholesterol crystals in 2 mls. of chloroform in a test-tube. Add one volume of concentrated sulphuric acid. Let stand and note the colored layer of chloroform floating on the acid solution. Pour into an evaporating dish. A variety of colors will appear. Transfer a small portion back to a test-tube and add one-half volume of water. Note that the color disappears. Add one-half volume of concentrated sulphuric acid and note the return of the color. Add a portion of glacial acetic acid and note the reddish color imparted. The reaction is due to the condensation of the aromatic ring by the sulphuric acid to form colored substances.[6] The reaction may be produced by using glycerol, which gives a more

[1] Paraffin-oil may be used, but lanolin prevents the tissues from becoming dry.
[2] Digitonin from digitalis, used to treat broken compensation in the heart.
[3] Chapter XIV. Ergosterol is referred to.
[4] Greek *kopros*, dung, *stereos*, solid.
[5] Salkowski, E., Berlin chemist, eminent in biochemistry. See portrait, page 216.
[6] Page 171.

characteristic color: The chloroform extract of cholesterol is treated with a drop or two of glycerol and this solution is layered with sulphuric acid; a pink to magenta color is developed.[1]



Ernst Salkowski, Berlin chemist, eminent in biochemistry. (From Garrison, History of Medicine.)

Liebermann's[2] reaction: The principle of this test is the acetylation of cholesterol[3] by means of acetic anhydrid and then condensation to the colored substance of the preceding reaction. Procedure: Place a few crystals of cholesterol in a dry test-tube and add 3 mls. of chloroform. To this solution add about 10 drops of acetic anhydried, $CH_3.C(=O)\backslash O /(=O)C.CH_3$, and drop into the mixture, one by one, about 3 drops of concentrated sulphuric acid. Mix the solution thoroughly and a deep blue color will develop.

Chemical Characteristics of Cholesterol.— Cholesterol is a cyclic terpene or, more definitely, member of the phenanthrenes:

Phenanthrene.

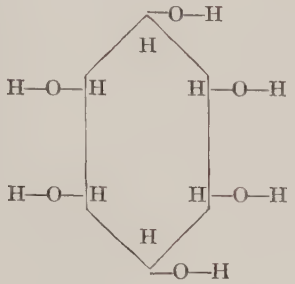
A group found in coal-tar and similar compounds and radicles occurs in plants of rather wide distribution. To this group the general term

[1] Read, B. E., and Meleney, F. L., Cholesterol: Its Occurrence in Two Thyroid Cysts; a Modification of the Salkowski Test, China Medical Journal, March-April, 1923.

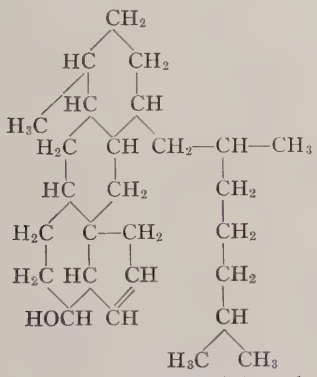
[2] Liebermann, A., Austro-Hungarian physician.

[3] The acetylation is done by way of the secondary alcohol radicle; see formula, page 217.

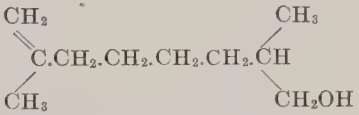
Cycloses was given by Lippmann.[1] One of the most common of these aromatic compounds is inosite which has the same empirical formula as glucose, but is entirely different in composition, being a hexa-hydroxy-benzene:



In the animal kingdom it occurs in heart tissue and in brain; in the plant kingdom, in legumes like peas and beans, before germination. Cholesterol is associated in plants with such members of the aromatic series as we have mentioned. Its chemistry is not entirely clear, but the following formula represents fairly accurately the modern concept of the molecule:



Windaus' formula for cholesterol.



A terpene (oil of citronella).

The formula of a well-known terpene is shown to the right of that of cholesterol.

The characteristic features of the cholesterol formula are:

(1) The terpene configuration.

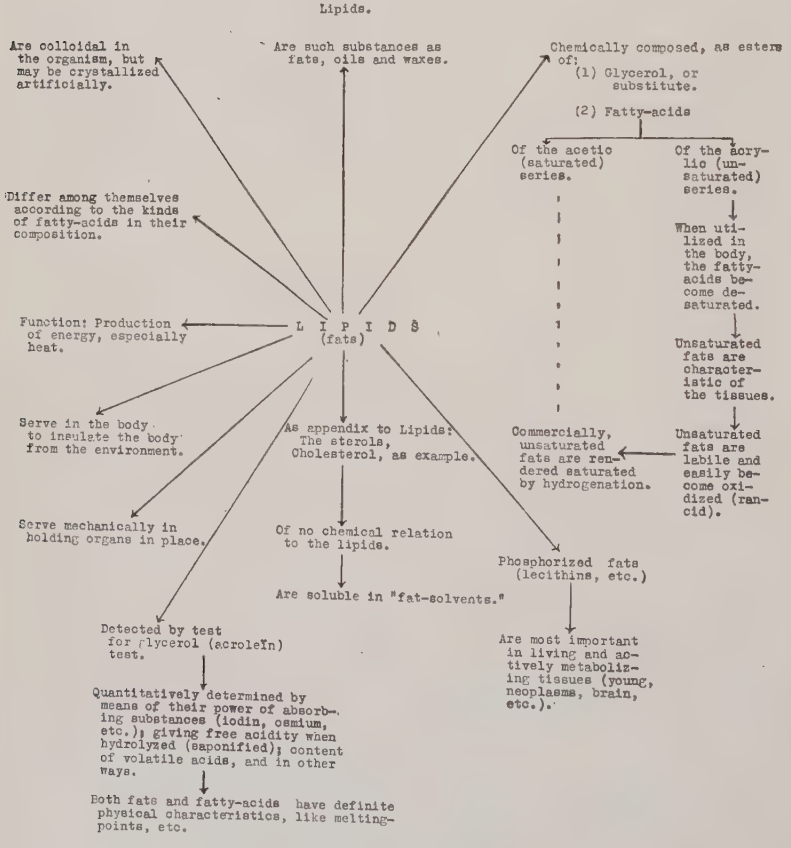
(2) The unsaturated group, CH_2, terminating the left-hand portion of the terpene, by which it is attached to the ring; this is a common characteristic of terpenes (compare citronella).

[1] Lippmann, A., German chemist.

(3) The secondary alcohol group, HCHO, which is acetylated in the Liebermann reaction given above (page 216). This secondary alcohol radicle is responsible for calling cholesterol an alcohol.

Bloor believes that much of the digested fat, especially the unsaturated fatty acids, is carried through the blood-stream as cholesterol esters, the esterification being accomplished by way of the secondary alcohol. Other than this and the rôle of cholesterol in the matter of photic effects, mentioned before (page 215), nothing is known concerning the participation of cholesterol in the economy of the body. Indeed, until recently cholesterol has been considered as a by-product and as something for the body to eliminate as best it can. The possibility that cholesterol and its allies, passing through the blood of the skin

GRAPHIC SUMMARY


Lipids.
Are colloidal in the organism, but may be crystallized artificially.
Are such substances as fats, oils and waxes.
Chemically composed, as esters of:
(1) Glycerol, or substitute.
(2) Fatty-acids
Of the acetic (saturated) series.
Of the acrylic (unsaturated) series.
When utilized in the body, the fatty-acids become desaturated.
Unsaturated fats are characteristic of the tissues.
Unsaturated fats are labile and easily become oxidized (rancid).
Commercially, unsaturated fats are rendered saturated by hydrogenation.
Differ among themselves according to the kinds of fatty-acids in their composition.
Function: Production of energy, especially heat.
L I P I D S (fats)
Serve in the body to insulate the body from the environment.
As appendix to Lipids: The sterols, Cholesterol, as example.
Serve mechanically in holding organs in place.
Of no chemical relation to the lipids.
Phosphorized fats (lecithins, etc.)
Are soluble in "fat-solvents."
Are most important in living and actively metabolizing tissues (young, neoplasms, brain, etc.).
Detected by test for glycerol (acrolein) test.
Quantitatively determined by means of their power of absorbing substances (iodin, osmium, etc.); giving free acidity when hydrolyzed (saponified); content of volatile acids, and in other ways.
Both fats and fatty-acids have definite physical characteristics, like melting-points, etc.

may act as a transmission mechanism for photic energy to become carried internally, is interesting and perhaps of far-reaching importance.[1]

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[1] Page 559.

CHAPTER VI

STRUCTURE-PRODUCING SUBSTANCES: PROTIDS

"Proteins are very much the same. Yet each animal and plant is different. Some constituent is evidently placed in a different position and the general mixture is open to all sorts of variations."—*Plimmer.*[1]

Nitrogenous Compounds.—In the preceding chapters we have discussed substances which have the elements carbon, hydrogen, and oxygen.[2] We shall now consider compounds which contain these three elements and an additional one, nitrogen. These substances are the protids,[3] so called because they are regarded as fundamental in the composition of protoplasm. It is still a question what relation exists between protids and the life processes, but this much is certain: The substratum of vital activities everywhere contains protids.

The Chemical Structure of "Protoplasm."[4]—Protids as well as glucids and lipids have bonds which serve to link them all together into a protoplasmic unit. There are theoretical reasons based on laboratory experience, however, to weigh against this view; these reasons will be discussed later.[5] The term "protoplasm" has no significance in chemical physiology, but serves simply as a descriptive expression for that material accompaniment of life, or, as Huxley expressed it, "The Physical Basis of Life." It is colloidal in nature and chemical analyses show that it has a variable constitution, as might be expected from a mixture of different substances. Nevertheless, some physiologists have believed that protoplasm is composed of one large molecule, known as a "biophore," "biogen,"[6] etc., which is very labile, that is, it readily undergoes change. The modern con-

[1] Plimmer, R. H. A., Professor of Biochemistry, St. Thomas Hospital, London. See Practical Organic, and Biochemistry, New York, Longmans, Green & Co., 1923. For an account of this subject see Lloyd, D. J., Chemistry of the Proteins and Its Economic Applications. Philadelphia, P. Blakiston's Son and Co., 1926.

[2] With the exception of the nitrogenous portions of the lipids, page 211.

[3] Greek *protos*, first. Named proteïns by Mulder; see Mendel, L. B., Nutrition (cited on page 594), pp. 15, 16.

[4] Von Mohl called the slimy material found in living substances *protoplasm*, which was named sarcode (Greek *sarx*) by the French savant, Dujardin.

[5] Page 304.

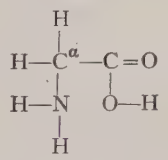
[6] Greek *bios*, life, *phore*, carry. Greek *bios*, life, *gen*, produce.

ception of protoplasm is virtually that of Hofmeister,[1] namely, that the cell is a little laboratory where various chemical reactions take place, the reactions confined to some distinct part of the cell by means of its physical structure. It has been pointed out by Hopkins[2] that the place of essential action of vital activities of the cell is at the great interface, the nuclear membrane which separates the nuclear plasma from the cytoplasm.[3] The peculiar reactions in colloidal solutions take place at the interfaces and not in the interior of the particles, and probably vital functions are similar. In fact, this organization of the unit of living matter into cells, etc., is one of the great characteristics of life, wherever found.

CHEMICAL STRUCTURE OF PROTIDS

If we select protid from several sources, such as meat, cheese, or beans, and subject it to chemical analysis, it is found that the chemical structure is similar, regardless of the source. In our study of the glucids we found that the higher and more complex compounds are capable of being built up of simple sugars, like glucose. In our discussion of the fats a similar construction was pointed out, fatty acids and higher alcohols being combined to form the more complex fats. Now in the protids we have a repetition of this building up of complex substances from simpler ones. The chemical principles involved in analyses and syntheses are similar in all three instances, for synthesis involves the abstraction of water, while analysis, or breaking down, is a matter of furnishing water for the process of hydrolysis.

Amino-acids: the End-product of the Protids.—The nitrogen of the protid exists in the form of ammonia incorporated into a fatty acid. This ammonia radicle NH_2, known as the amino-group, enters the fatty acid in the alpha position, that is, on the carbon atom preceding the carboxyl. Alpha-amino-acetic acid, the simplest of the amino-acids and derived from acetic acid, illustrates this point:



Alpha-amino-acetic acid or glycin.[4]

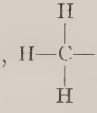
[1] Hofmeister, F., German physiologist and biochemist, founder of important biochemical journals, professor at Strassbourg. See Hofmeister, F., Die chemische Organization der Zelle, Braunschweig (Brunswick), 1901.

[2] Hopkins, F. G., Sir Professor of Biochemistry, Cambridge University, England; discoverer of vitamins, glutathion, etc.; foremost British biochemist.

[3] The protoplasm outside the nucleus (Fig. 52).

[4] Also called glycocoll. The alpha carbon is shown at α.

All the amino-acids which enter into the composition of protids are alpha-amino-acids.[1] Beta-amino-acids are unstable and do not enter into the composition of protids and gamma- and delta-amino-acids never form a part of the protid, although they may be found in connection with living activities, as in the fermentation of muscle, gelatin, etc., in which they are derived from α-amino-acids.

The Structure of the Amino-acids.— One of the hydrogens of the methyl group,  , in a fatty acid may be replaced by one of a number of atoms, radicles, etc., such as an halogen atom, an hydroxyl, an alkyl group,[2] or by ammonia in the form of the radicle NH_2, in which case an amino-acid is formed. The introduction of ammonia into a fatty acid is easily accomplished in the laboratory by heating monohalogen fatty acids, like monochloracetic acid, $CH_2.Cl.COOH$, with ammonia; the ammonia replaces the halogen, chlorin, to form glycin, the substance mentioned above:

$$\underset{\text{Monochloracetic acid.}}{CH_2.Cl.COOH} + \underset{\text{Ammonium hydroxid.}}{NH_2.\boxed{H_2.O}H} = \underset{\text{Glycin.}}{CH_2.NH_2.COOH} + H_2O + HCl.$$

Other radicles, like the benzene and other rings, may be incorporated into amino-acids, but none of these affects the position of the amino-group.

Protids of Blood.[3]— The protids of the blood may be used as practical examples. They are useful in the diagnosis and differentiation of certain diseases. Interesting theoretical considerations concerning them also arise. For example, under certain pathological conditions protids appear in the urine. What is their origin? Are they derived from the blood?

Preparation of Blood-serum for Study.—The clear, yellowish liquid exuding from a bleeding wound after clotting has taken place is blood-serum. Quantities of blood may be obtained from slaughter-houses and if such blood be placed in tall cylinders and permitted to clot, the serum will collect at the top of the blood column, the clot being withdrawn downward by gravity. This serum is pipetted out and diluted with distilled water, one part of serum to nine portions of water. Note that some of the constituents of the serum are precipi-

[1] The possibility of β-alanin is discussed on page 373.

[2] Like methyl, ethyl, etc.

[3] For an account of blood protids see Howe, P. E. The function of the plasma protide. Physiol. Revs., vol. 5, page 439, 1925.

tated; these constitute the globulin fraction, the albumin[1] remaining in the filtrate from the diluted serum. The diluted serum must be filtered, both portions being saved.

Exercise 1.—*The Characteristics of the Globulin Fraction.*—To 5 mls. of the filtrate, mentioned above, which contains dissolved globulin,[2] add 5 mls. of a saturated solution of ammonium sulphate[3] and let stand for ten minutes. Note the additional precipitate of globulin. Filter this off, washing once with 5 mls. of distilled water and save both fractions. Proceed, now, to the study of the globulin. Scrape a small amount from the filter-paper and suspend it in distilled water in a test-tube. Note whether it goes into solution or not.[4] If it does not go into solution, add, drop by drop, some of the ammonium sulphate solution, shaking the tube after each addition of the salt solution and noting when the globulin begins to dissolve. Globulin is insoluble in distilled water, but soluble in dilute salt solutions. It is insoluble in half-saturated solution of ammonium sulphate. Try the effect of adding crystals of magnesium sulphate to a solution of globulin in a dilute salt solution (NaCl). Note that blood-serum is insoluble in fully saturated solution of magnesium sulphate, $MgSO_4$.[5] By adding saturated solution of sodium chlorid[6] to a suspension of globulin in distilled water, pseudo-globulin is dissolved, which may be again precipitated with half-saturated solution of ammonium sulphate. Globulin prepared by simple precipitation as in the first part of the exercise, is called true, or euglobulin, to distinguish it from the pseudo-globulin which is soluble in distilled water and also in concentrated (saturated) sodium chlorid solution. Pseudo-globulin is probably a protected colloid,[7] lecithin or cholesterol being the protective agent. The toxicity of stronger solutions of sodium chlorid is due to the physical rather than to chemical actions, namely, alterations of the water content of the colloidal protid.[8]

[1] Note the spelling; the term ending in *-en* is synonymous with protid, but the term "albumin" is the technical one for water-soluble protid described above.

[2] Only a part of the globulin is precipitated from distilled water. There is enough salt adhering to the protid to keep some globulin in solution.

[3] At room temperature, 80 gs. solid, crystalline $(NH_4)_2SO_4$ to 100 mls. of H_2O.

[4] If it does, there is sufficient ammonium sulphate adhering to cause it to do so, in which case wash the original globulin on the filter-paper more thoroughly with distilled water.

[5] 102 gs. $MgSO_4$ to 100 mls. distilled water.

[6] 36 gs. NaCl per 100 mls. H_2O.

[7] Page 128. From pseudoglobulin, euglobulin may arise. For a discussion of the relations of albumins and globulins see Hartley, P., Biochem. J., vol. 8, p. 541, 1914.

[8] Page 128.

Exercise 2.—*Coagulation of Globulin.*—(1) By heat: Transfer 5 mls. of a solution of globulin in 1:10 NaCl solution to a test-tube and acidify with a drop of glacial acetic acid. Place the tube so that the bottom is about 1 cm. above the tip of the flame of a micro-burner and watch carefully for evidences of coagulation. Repeat, omitting the addition of acid. Free hydrogen ions (acidity) are necessary for heat coagulation. See next Exercise for evidence that an electrolyte is necessary also.

(2) By alcohol: To 5 mls. of the solution made as directed in the previous section, add 95 per cent. ethanol, drop by drop, until a cloudiness is visible in the solution of globulin, agitating the tube as the additions are made. Now filter off the precipitate, wash with distilled water, and scrape some of the solid from the paper. Transfer the scrapings to a test-tube containing 1:10 NaCl solution and determine whether they go into solution. The brief contact with alcohol causes precipitation. Repeat this experiment, but leave the protid in contact with the alcohol over night and then test for solubility as before. The prolonged exposure of protid to alcohol causes irreversible coagulation.

Exercise 3.—*Globulin by Dialysis.*—See page 106 for the method of procedure for dialysis of a protid. Using the suspension of globulin obtained by half-saturating the diluted blood-serum with ammonium sulphate, dialyze it against running tap-water over night, or until the following period. Collect some of the globulin thus prepared and test its solubilities. Test its ability to undergo coagulation, referring to the previous Exercise; for coagulation requires not only acid reaction but also the presence of an electrolyte, such as an inorganic salt.

In cooking eggs salt is added unless the frying is in bacon grease, when the bacon introduces sufficient salt. The better cooks in poaching eggs also add a drop of weak vinegar (acetic acid) in order that the egg-white may be coagulated evenly and not become toughened around the edges.

Practical Applications.—In the diagnosis of renal disease (nephritis), it is necessary to differentiate between two principal types of lesions: Interstitial and parenchymatous nephritis. The former involves a retention of various blood constituents; the latter is characterized by the loss from the blood of protid which passes off in the urine. Robertson,[1] found that in interstitial nephritis globulin

[1] Robertson, T. B., Professor of Biochemistry, Adelaide, South Australia. See Principles of Biochemistry (citation, page 196), p. 339.

accumulated in the blood to the extent of 25–50 per cent. of the total protid (albumin and globulin), the average normal ratio being from 16–35 per cent. of the total protid. In chronic parenchymatous nephritis, although the total protid is greatly reduced in the blood and increases in the urine, the globulin is greatly increased in the blood:

	Total protid per cent.	Globulin per cent.
Normal	7.4 gs.	37.0 gs.
Chronic interstitial nephritis	6.7 "	35.7 "
Chronic parenchymatous nephritis	3.9 "	89.2[1] "

Robertson devised a method for estimating the various portions of the protid of blood by refractometry, which requires only about 1 ml. of serum for a determination. The importance of diagnosis of a particular form of nephritis is evident when we consider that the method of treatment, feeding, etc., is quite different for the two forms of the disease. Besides nephritis, other diseases exhibit differences in the ratio of globulin to total protid and to albumin, infections and toxemias causing a typical rise in the proportion of globulin. The globulin content of spinal fluid is a reliable diagnostic agent. Again it has been shown that a relation exists between the globulin content of the blood and the age of the animal. Howe[2] found that the newborn calf before beginning to feed from the mother lacked globulin in the blood, but that after the colostrum[3] had been taken in during the first feeding from the mother's teats, the globulin ratio rapidly rose. Quite interesting is the correlation between colostrum and immunity from infection in calves, those receiving colostrum being much less susceptible to infections than those from which colostrum was withheld.[4] However, for the human infant this relation does not seem to hold[5] as definitely as for the calf.

It is interesting, likewise, that ultraviolet light of 2900 Å is capable of converting albumin into globulin; such a factor may be responsible for the curative effect of such light in tuberculosis and other diseases. Globulins differ from albumins in the fact that they are difficult to crystallize.

[1] After Epstein, A. A., Mount Sinai Hospital, New York. See Article, Amer. Jour. Med. Sci., vol. 154, p. 638, 1917.

[2] Howe, P. E., U. S. Department of Agriculture, Washington, Jour. Biol. Chem., vol. 49, p. 115, 1921.

[3] Casein-poor secretion first secreted by the mammary glands.

[4] Smith, T., Director of the Rockefeller Institute Department of Animal Pathology, Princeton, New Jersey. See Jour. Exp. Med., vol. 36, p. 181, 1922.

[5] See Kuttner, A., and Ratner, B., Amer. Jour. Diseases of Children, vol. 25, p. 413, 1923.

Exercise 4.—*The Characteristics of the Albumin Fraction.* Using the filtrate saved from the first filtration of blood-serum,[1] the filtrate being a half-saturated ammonium sulphate solution containing dissolved albumin, add solid ammonium sulphate crystals to complete saturation; albumin is precipitated. Filter it from the solution, washing with 2 mls. H_2O, and use it in the following tests:

Exercise 5.—Scrape some from the paper and test its solubility in distilled water, in a 1:10 NaCl solution and try to dialyze some against distilled water. Does it dialyze? Can you recover albumin as you did globulin by dialysis? Can you cause heat coagulation of albumin as you did globulin? Aside from solubility, the reactions of albumin and of globulin are similar.

Exercise 6.—Precipitate the globulin from about 25 mls. of serum diluted 1:10 with distilled water, filter, and then determine whether you can precipitate the albumin by (1) Saturating the filtrate with solid magnesium sulphate, or (2) by adding one volume of saturated ammonium sulphate to one of the filtrate. Repeat (2), using (*a*) Filtrate which has had a drop of 10 per cent. NaOH solution added to it, and (*b*) filtrate which has had a drop of decinormal sulphuric acid added to it. Neither saturated $MgSO_4$ nor half-saturated $(NH_4)_2SO_4$ in neutral or in alkaline reaction causes precipitation of albumin, but precipitation occurs in acid medium.

Exercise 7.—*The Temperature of Coagulation of Serum Protids.*—Make a dilution of serum 1:10 and filter. Add a drop of 1:10 HCl. Draw out a small bulb upon a glass tube, permit the tube to cool, and then suck the diluted filtrate into the bulb.[2] Break the tubing from the upper part of the bulb at a distance of about 2 cms. and seal the end in a low flame. Permit the glass to cool in an inverted position, invert the bulb, and, after the liquid has drained from the extension of the bulb, seal it as you did the upper end. Let the tube cool before re-inverting it. Now attach the bulb containing the filtrate to the bulb of a thermometer by means of two narrow rings of rubber tubing. Lower the thermometer into a beaker of water and fasten the thermometer in position by means of a ring-stand and clamp. Place this beaker in a second one containing enough water to become level with that in the first beaker. Place the larger beaker on a wire gauze over a small flame and carefully determine the point at which coagu-

[1] Page 223.
[2] Figure 227.

lation of the serum protids occurs as indicated by the developing cloudiness of the solution within the bulb. If bubbles on the surface of the beakers interfere with observations of the bulb, remove the bubbles by means of a rubber-tipped glass rod, commonly called a "policeman."

It will be found that a 10 per cent. solution of serum is coagulated by heat at $75 \pm 5°$ C. Egg-white coagulates at a lower temperature—about 62° C.

Serum protid resembles egg-white in responding to various electrolytes in different ways when undergoing coagulation, depending upon electrochemical relations. If protid is in an acid medium, it becomes electropositive as explained on page 108; the protid behaves as a cation and makes salts of the nature of protid-chlorid. The anion is the effective agent in producing precipitation, and it is the valence of the anion, not the kind that is effective.[1] Monovalent ions, like Cl^-, are less effective than bivalent anions, like $SO_4^=$. The reason why protids are precipitated to a greater extent by bivalent and polyvalent ions than by monovalent is because the monovalent ion permits water absorption by the protid; this supports the protid in suspension. On the other hand, polyvalent ions, like $SO_4^=$ and $PO_4^=$, give a smaller number of ions to the protid; the chlorion requires one protid cation to unite with it, the sulphion two, and the phosphion three. Hence it will require one-third as much $PO_4^=$ to cause precipitation as Cl^-.

Clinical Application.—Determination of the time necessary for the blood of a patient to clot spontaneously is a routine procedure in hospital work because of its influence on surgery. Fibrinogen is a globulin-like protid in the blood which becomes converted into fibrin under special conditions.[2] Two chief methods for the determination of blood-clotting time are in use:

(1) *The Method of Howell.*[3]—Draw 5 mls. of blood as directed on page 67, but without the use of any anticoagulant or preservative. Transfer to a clean test-tube and determine the time necessary for a

[1] The reader may encounter the so-called "Hofmeister Series" of anions arranged according to their supposed effect. Loeb showed that if the reaction of the solution of protid is not permitted to change, all anions of a given valence work alike. See Loeb, citation, p. 129.

[2] Page 317.

[3] Howell, W. H., Director of the School of Hygiene and Public Health, Johns Hopkins University, formerly Professor of Physiology in the Medical School and author of the well-known text-book, Text-book of Physiology, Philadelphia, W. B. Saunders Co., 1924, 9th ed.

clot to form, so that the tube can be inverted without causing any flow of blood. The average time by this method is five minutes.

(2) *Method of Wright.*[1]—Draw out, from a 2 mm. glass tube with heavy walls, capillary tubes about 2.5 cms. long. Cool and draw up into them blood freshly drawn as described in (1). Make the tubes into a bundle by means of two rubber bands, one broad enough to close the ends of the tubes as the band is passed around them lengthwise. Drop the bundle into a water-bath at the constant temperature of 37° C. (blood temperature). At the end of one, two, three, four, and five minutes withdraw a tube and determine whether the blood has coagulated within it by attempting to blow through it. The average normal coagulation time by this method is 3.5 to four minutes. In certain physiological states, like hunger, hemorrhage, transfusion, etc., the clotting time is diminished. In pathological cases of "bleeders" (hemophilia), ordinary obstructive jaundice, asphyxia, snake-bite, and in certain hepatic disorders the clotting time is increased; the blood of the congenital[2] hemophiliac coagulates only after fifteen minutes.

Methods of Precipitating Protid from Solution.—These may be divided into the following groups: (1) Methods involving the use of salts of the heavy metals—lead, mercury, iron, etc., and (2) those involving the use of "alkaloidal reagents."

(1) *Methods Involving Use of the Salts of the Heavy Metals:*

EXERCISE 8.—Lead acetate, $\begin{matrix} CH_3COO \\ \diagdown \\ \quad\quad Pb \\ \diagup \\ CH_3COO \end{matrix}$, and a mixture of lead acetates formed by boiling litharge, PbO, with lead acetate[3] are used frequently to precipitate protid from solution. They give white precipitates of protids of the coagulable series, albumin and globulin.

Try the effect of adding a few drops of (1) Lead acetate and (2) "basic lead acetate" to test-tubes containing protid solutions. Filter. The filtrates should remain clear if there has not been added an excess of acetate. For the preparation of clear filtrates for polariscopic work[4] lead acetate is used to advantage, but basic lead acetate should not be used in this case.

[1] Wright, A. E., English physician and bacteriologist. See Technic of the Blood-test and Capillary Glass Tube, London, Constable, 1921.

[2] From birth.

[3] This mixture is known as "basic lead acetate."

[4] Page 183.

Exercise 9.—Mercuric chlorid, $HgCl_2$, precipitates protids in neutral, aqueous solutions. Repeat the foregoing Exercise, using a drop or two of a solution of mercuric chlorid. Before filtering add some saturated solution of sodium chlorid or some solid crystals of this salt. The precipitate dissolves because sodium is more active, chemically, than mercury.[1] Add dilute hydrochloric acid; the precipitate reappears. Precipitation with mercury proceeds best in a slightly alkaline reaction, when the protid is electronegative; Hg^+ then unites with the negatively charged protid-anion.

The antiseptic use of mercury ("corrosive sublimate") is due to the precipitating action of the salt upon the protids of bacteria. Its action is slow, and for this reason $HgCl_2$ has given place to other antiseptics for certain purposes. In mercuric chlorid poisoning death ensues if the mercury compound is permitted to reach the kidney in sufficient amount to damage that organ severely. Anuria[2] and retention[3] ensue.[4]

Exercise 10.—Add to a solution of albumin, ferric chlorid solution (5 per cent.) drop by drop. Note the precipitate. Continue adding iron solution; note that the precipitate disappears. Protid is soluble in excess of ferric chlorid solution.

Exercise 11.—Cupric sulphate precipitates protid, the color of the precipitate being characteristic; it is bluish violet, which is the basis of the chief color reaction of all true protids.[5] The copper-protid compound dissolves in a 10 per cent. solution of NaOH, giving a violet color which is intensified on standing.

The toxicity of copper compounds obtained in cooking vegetables in copper vessels is due to the precipitating action of the copper substances upon the protids of the alimentary tract and perhaps also of the kidney.

Cupric sulphate is used to kill plants which grow in swimming-pools and reservoirs and it is efficacious in killing bacteria.

[1] Mercury has a tendency to gain electrons and to become metallic mercury.

[2] Greek *a*, privitive, meaning without, and *ouria*, urine. In a case in Jefferson Hospital only 4 mls. of urine were passed in seven days.

[3] This term signifies that substances like phosphates, chlorids, and nitrogenous substances do not pass through the kidney, but are retained in the blood-stream. In the case just mentioned the normal amount of nitrogen (25 mgs. per 100 mls. blood) rose to 200 mgs.

[4] For an account of mercuric chlorid poisoning in several cases see Looney, J. M. Journ. Biol. Chem., vol. 70, page 513, 1926.

[5] Page 285.

Aluminium, in the form of alums, exerts a double effect upon protids: The compounds are frequently in a colloidal state and precipitate protids. This property is utilized in water purification; the protids contained in the water are precipitated as the water comes into contact with lumps of alum and is deposited in precipitation tanks, the supernatant water being free from such contaminations. The second action of aluminium compounds is an astringent action, by virtue of chemical reactions between the aluminium and the protids. Alum is used in some baking powders; the sulphate acts upon bicarbonate freeing CO_2 which, rising through the gluten of the dough, causes the "leavening" (Latin *levis*, light); on baking, the interstices in the dough are fixed by the heat into permanent cavities. Alum is used either as the ammonium, sodium or potassium salt. Using sodium alum, we have the following as a typical reaction:

$$Na.Al(SO_4)_2 + 3NaHCO_3 = 2Na_2SO_4 + Al(OH)_3 + 3CO_2.$$

In other baking powders, different means of decomposing and forming CO_2 are employed:

With tartaric acid:

$$HOOC.CHOH.CHOH.COOH + NaHCO_3 = K.NaC_4H_4O_6 + H_2O + CO_2$$

With "cream-of-tartar": (potassium hydrogen tartrate)

$$HOOC.(CHOH)_2.COO.K + NaHCO_3 = K.NaC_4H_4O_6 + CO_2 + H_2O.$$

With phosphate: (calcium hydrogen phosphate)

$$CaH_4(PO_4)_2 + 2NaHCO_3 = CaHPO_4 + Na_2HPO_4 + CO_2 + H_2O.$$

In the alum powders, aluminium carbonate, $Al_2(CO_3)_2$, is an intermediate step not indicated in the reaction given above since it is hydrolyzed to the hydroxid:

$$Al_2(CO_3)_3 + 6H_2O = 2Al(OH)_3 + 3H_2O + 3CO_2.$$

The hydroxid is insoluble in water but is soluble in the hydrochloric acid of the stomach. The question as to the absorbability into the blood of aluminium and the problem as to the toxicity of alum is still an open one. The reader is referred to Petersen, Haines and Webster[1] and to Underhill's Toxicology.[2] Alum is used in the "styptic sticks"[3] employed by hair dressers to inhibit the bleeding of wounds.

[1] Petersen F., Haines, W. S. and Webster, R. W. Legal Medicine and Toxicology, Philadelphia, W. B. Saunders Co., 1923 second ed., page 165; also Underhill, F. P. Toxicology, Philadelphia, P. Blakiston's Son and Co., 1924, page 116.

[2] See Cushny, A. R., Text-book of Pharmacology and Therapeutics., 8ed, 1924.

[3] Greek *stupho*, contract; compare the word "stop." The use of the styptic stick has been forbidden by law, since the antiseptic action of these preparations is not high enough to insure that infection is not transmitted from customer to customer.

(2) *Methods Involving the Use of "Alkaloidal Reagents."*—These substances (picric acid, etc.) have long been used for precipitating "alkaloids," basic substances like nicotine of the pyridin group; cocaine of the tropane group; quinine of the quinoline group, and morphine of the iso-quinoline group. These alkaloidal reagents give negative ions and precipitate the basic, positively charged alkaloids, as well as protid in electro-positive condition, as in acid medium. The alkaloidal reagents are presented in practical form here:

Exercise 12.—Acidify a protid solution with a few drops of glacial acetic acid and add a drop or two of 5 per cent. solution of potassium ferrocyanid, $K_4Fe(CN)_6$. Hydroferrocyanide, $H_4Fe(CN)_6$ is formed, which precipitates the positively charged protid as a whitish substance. A blue color may be imparted to this precipitate and to the solution by boiling the preparation, which causes the decomposition and oxidation of some of the ferrocyanid to form Turnbull's blue, $Fe_3[Fe(CN)_6]_2$.

Exercise 13.—Acidify 5 mls. of a protid solution with a drop of dilute acetic acid and then add a few drops of saturated picric acid (2 per cent.). The precipitate is yellow.

Exercise 14.—*Esbach's*[1] *Reagent.*—This is a modification of the preceding reagent. Citric acid is added to a 1 per cent. solution of picric acid in the same amount (1 per cent.). To a protid solution add one-half its volume of Esbach's solution;[2] the precipitate is yellow as before.

Quantitative determination of protid by Esbach's method: Obtain an Esbach tube (Fig. 225) which resembles a test-tube, but has graduations in tenths of per cent. and also two marks, "U" and "R," signifying the level to which the protid solution[3] is filled and the "R," the level to which the reagent is added. Using a protid solution, fill to the mark "U" and add the reagent to the mark "R." Insert the rubber stopper accompanying the tube and invert it to permit mixing of the two solutions. Let the tube stand until the next period, or over night. Read the upper level of the precipitate as parts per liter or tenths of per cent. coagulated protid.

[1] Esbach, C. H., French physician, Paris, 1843. See Chapter XV.

[2] The composition of the reagent is given in Appendix.

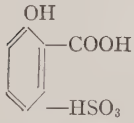
[3] The tube was designed for urinary analysis and the "U" refers to urine.

Exercise 15.—To a protid solution add tri-chlor-acetic acid, CCl_3COOH, 5 per cent. solution. The precipitate is whitish. This is a valuable method for causing the precipitation of protid.[1]

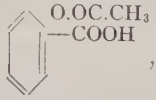
Exercise 16.—To a protid solution add a few drops of dilute HCl solution and then some tannic acid, best in the form of Almén's solution[2] an acid-alcohol preparation. Note the precipitate.

Exercise 17.—To a protid solution add a few drops of fresh 25 per cent. solution of meta-phosphoric[3] acid. White precipitate. An old solution of this reagent is composed chiefly of phosphoric acid that is incapable of precipitating protid.[4]

Exercise 18.—To a protid solution add a few drops of one-twelfth normal sulphuric acid solution and then of a 10 per cent. solution of sodium tungstate, $Na_2WO_4.2H_2O$. Note the precipitate of white protid. For the application of this precipitant see Chap. XVI.

Exercise 19.—To a protid solution add some dry sulphosalicylic acid, 

; the precipitate is white.

Other salicylates cause the precipitation of protid. Examples are salicyclic acid, used in preserving fruits and vegetables; aspirin, acetyl-salicylic acid, 

, an analgesic[5] used in medicine since 1899 for the purpose of lowering temperature in fever. Its first use was founded upon an error, for it was supposed that it acted as an antiseptic without the irritating effects of antiseptics of

[1] The method of Quick (Chapter XV) utilizes this reagent.

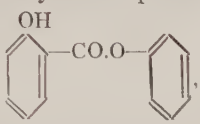
[2] Appendix.

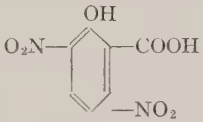
[3] The difference between the three kinds of phosphoric acid (H_3PO_4) is the degree of hydration. Metaphosphoric acid may be considered to be $P_2O_5 + H_2O = 2HPO_3$; the ion is PO_3^-. See page 73.

[4] For an account of an intensive study of protid precipitants, see Van Slyke, D. D., Jour. Biol. Chem., vol. 53, p. 253, 1922.

[5] Greek *ana*, against; *algos*, pain. Compounds like these were formerly known as antipyretics (Greek *anti*, against, *pyr*, fire). The function of such substances is to lower body temperature, but since the action is due to the effect upon the blood vessels of the skin, which become dilated, thus bringing blood to the surface, and increasing perspiration, which cools the body, the former term is preferable, for analgesics do not affect the heat mechanism directly; the effect is on the heat-dissipating mechanism, evaporation.

similar potency, like benzoic acid, etc. Hanzlik,[1] however, found that its toxicity is distinct, while the antiseptic action is limited as used. It should be employed only when prescribed by a physician.

Salol, or phenyl-salicylate,  , was introduced as an intestinal antiseptic in diseases of the lower alimentary tract, it being supposed to pass through the stomach unchanged, but to be decomposed in the intestinal alkalinity into phenol, which gave the antiseptic action. However, the toxicity is high, owing to the ready absorption of the phenol by the walls of the intestine, leading to necrosis and extensive irritation of the tracts.

Di-nitro-salicylic acid,  , is used by Sumner[2] to precipitate protids in certain reactions.

THE HYDROLYSIS OF PROTIDS

Exercise 20.—To 25 mls. of 10 per cent. protid solution add 250 mls. of 2:1000 solution of hydrochloric acid. Stopper with cotton wool and leave for twenty-four hours or until the next period. Proceed with the study of the metaprotids.[3]

Exercise 21.—Acid albuminate. Using 25 mls. of the HCl-treated solution, add 2 per cent. Na_2CO_3 to neutralization with litmus and note the flocculent precipitate of acid albuminate. If one desire maximum yield, the iso-electric point[4] must be established (*p*H 4.7–5.4). Filter through glass-wool. Suspend the residue in the wool in 25 mls. of distilled water and use this preparation for the following studies:

Exercise 22.—To 3 mls. of the suspension, add, drop by drop, 4 : 1000 HCl solution; note that the precipitate dissolves after the addition of a small amount of the acid solution. Now neutralize the fluid containing the dissolved precipitate of metaprotid, using 1: 1000 solution of Na_2CO_3. The precipitate reappears.

[1] Hanzlik, P. J., Pharmacologist, Stanford University, San Francisco, California.

[2] Sumner, J. B., see page 24 and Chapter XV.

[3] Greek *meta*, next after, that is, a change; hence changed protids (changed by acid, etc.).

[4] Page 109.

Exercise 23.—Prove that metaprotid is soluble in excess of acid solution by adding to 3 mls. of the metaprotid suspension, concentrated HCl, drop by drop. This is the basis of the caution necessary in the acid (Heller's) test for protid in urine.[1]

Exercise 24.—Prove that an acid solution of metaprotid does not undergo heat coagulation.

Exercise 25.—Prove that an acid solution of metaprotid may be neutralized and alkali albuminate precipitated, the precipitate being soluble in excess of alkali.

Exercise 26.—Boil 3 mls. of the protid solution; a coagulum appears which is insoluble in acid solution. Metaprotid may be coagulated when in the form of a suspension of neutralized acid or alkali albuminate.

Exercise 27.—Prove that an acid solution of metaprotid can be salted out[2] with half-saturated ammonium sulphate solution, but that metaprotid in suspension cannot be salted out. This is a test for distinguishing globulins from metaprotids.

Exercise 28.—"Lieberkuehn's jelly."[3] Mix 5 mls. of undiluted egg-white with 2 mls. of twice normal solution of sodium hydroxid. Let stand and note the formation of a jelly, which is translucent and soluble in diluted salt solution. Prove that alkali albuminate (Lieberkuehn's jelly) cannot be converted into acid albuminate by adding acid to the jelly. The reason for this is that the protid molecule has been altered by the formation of the jelly; the sulphur of one of the amino-acids, cystin, is lost.[4]

In the above exercises we have studied the earlier changes which protids undergo when subjected to the influence of acids and alkalis. In all probability even water exerts an effect upon protid, and probably this effect is realized in the behavior of cells like leucocytes, or the freely living ameba. Apparently as the pseudopodia are formed, an alteration occurs in the protoplasm at the exposed portions of the ectoderm, as this part of the cell comes into contact with the water in

[1] Page 764. Nitric acid is used in this test, but the reaction is the same.

[2] Page 129.

[3] Lieberkuehn, J. N., a German anatomist of the 18th century.

[4] See Hammarsten, O., and Hedin, S. G. (Translated by J. H. Mandel, N. Y. University and Bellevue Medical School.) A Text-book of Physiological Chemistry, New York, John Wiley & Sons, 1917, 7th ed.

which the cells are immersed. At least there is a change in the dispersion of the colloidal portions when protid comes into contact with water, or with a salt solution, in which osmotic effects are present.[1]

Later Products of Hydrolysis.—We shall now proceed to the study of the later products of hydrolysis which are formed when protid is treated for some time with acid or alkali, or when the temperature is elevated. These late products correspond to the dextrins in glucid hydrolysis and are known as the albumoses, peptones, peptids, and amino-acids, or, collectively as the protidtemns.[2]

Exercise 29.—Preparation of protidtemns by acid hydrolysis of Hamburger steak: Obtain, from the storeroom, one kilogram[3] of Hamburger steak and suspend it in a pan in sufficient tap-water to cause it to make a hash suitable for experimental work. Carefully bring the temperature of the suspension to exactly 48° C. Strain off the water through scrim, or cheese-cloth, saving both portions. Return the residue of meat to the vessel and transfer the filtrate to a large evaporating dish. Add to the residue tap-water as before and bring the temperature to 56° C. Repeat the straining procedure, combining the filtrates and saving the residue. The third time, bring the water to the boiling-point. You have now obtained two of the protids of muscle, namely, myosin (47° C.) and myogen (56° C.), the former a typical globulin and myogen of the nature of albumin. Cool the combined extract in a refrigerator[4] in order to congeal the fat, which is then lifted off mechanically. Now place one-half the residue from the first part of the experiment in a flask and add 2:1000 HCl solution which has been mixed previously with dried pepsin,[5] thus making an artificial digestion mixture similar to gastric juice. Add enough toluene, , to make a thin layer over the surface of the fluid and then mix all the contents together. Leave the preparation in a

[1] Protid thus affected is called "denatured." See Wu, H., and Yen, D., Jour. Bioch. (Japan), vol. 4, p. 345, 1924.

[2] Protid plus Greek *temno*, to cut; the conception is that these products are cut from the molecule of protid. Similarly, we have lipidtemns and glucidtemns.

[3] Equivalent to 2.2 pounds. In crowded classes this Exercise may be conducted by two students working together.

[4] Or, during the colder weather, out-of-doors.

[5] If pigs' stomachs are available, carefully remove the mucosa and pass it through the coarser grinder of a meat-grinder; cover with glycerol and leave in a cool place. The glycerol absorbs pepsin, which may be used as a glycerol solution.

thermostat[1] at 37° C. for one week. In the meantime strain the superfluous liquid from the second half of the residual meat and suspend the residue in tap-water to which have been added 10 mls. of concentrated sulphuric acid for each 100 mls. of fluid. Arrange a condenser, preferably of the Hopkin's type[2] of reflex condenser to fit the mouth of a small balloon-flask[3] in which the protid solution may be boiled. Permit the contents to boil as vigorously as possible for 30 minutes. Now prepare to make a study of the contents of the flask.

Exercise 30.—*Scheme for Analysis of Hydrolyzed Protid.*—Filter. Then: (1) Remove 25 mls. of the acid solution and note carefully how many mls. of 10 per cent $Ba(OH)_2$ solution are necessary to cause neutralization to blue litmus-paper. Measure the total filtrate and add the appropriate amount of 10 per cent. $Ba(OH)_2$ solution to the whole, less 5 mls. Any metaprotid will be precipitated at this reaction. Filter, using glass-wool if necessary. Residue: Prove that the residue contains metaprotid. Filtrate: Go to (2).

(2) To the filtrate add one volume of saturated ammonium sulphate solution. Filter. Residue: This consists of the protidtemns known as albumoses. Go to (A). Filtrate: This consists of peptones; go to (B).

(A) Separation of albumoses: Suspend the residue from (2) in 2 volumes of 32 per cent. ethanol; use the proper strength of alcohol. A precipitate appears. Filter. Residue: Heteralbumose fraction. Test solubility in water, dilute acids, alkalis, and salt solutions (NaCl); $(NH_4)_2SO_4$; it is soluble in all but the first of these. Try heat coagulation. Prove that it may be salted out with half-saturated $(NH_4)_2SO_4$. Try the effect of adding nitric acid; warm the solution and note that the precipitate dissolves, but on cooling the test-tube under the cold watertap it reappears. The same reaction is obtained by using potassium ferrocyanid in acetic acid solution.[4] Note that heteralbumoses are precipitated by 5 per cent. solution $CuSO_4$. Filtrate:

[1] A thermostat for the purposes of such work may be made by securing two packing boxes, one somewhat larger than the second, into which the smaller box fits. Before placing the smaller box in the larger one make a layer of excelsior or, better, metal wool, upon which the smaller box rests. Then pack wool around the interspaces, leaving the top open to serve as a door. A small opening 20 cms. square may be cut in the top as a door. Then the rest of the top can be covered with the wool and the lid of the larger, outer box, nailed on. Heat may be furnished by a common incandescent lamp, a carbon filament giving more heat. One has the choice of several simple and inexpensive heat controls on the market.

[2] Fig. 112.

[3] Appendix.

[4] Page 231.

Protalbumose fraction. Using not over 5 mls. of the filtrate, precipitate the protalbumoses by adding 95 per cent. ethanol. The characteristics of the protalbumoses resemble those of the heteralbumoses, with the exception of their solubility in different strengths of ethanol.

(B) Proceed to the study of the pepton fraction: Go to (3). (3) To the filtrate from (2) add half its volume of saturated solution of ammonium sulphate. Any precipitate is filtered off, dissolved in water and treated with twice its volume of 80 per cent. ethanol. Residue: Thioalbumose. Filtrate: Saturate this fraction with crystals of $(NH_4)_2SO_4$; this contains syn-albumose; it may be obtained in precipitated form and purified by the use of ethanol. The filtrate contains peptons. These substances may be separated by concentrating the filtrate over a free flame to small bulk and then continuing the evaporation to dryness on a boiling water-bath. Take care that the preparation does not char. To the residue add small quantities of 95 per cent. ethanol and rub the mass with the end of a glass rod. Note the odor and color of meat extract. Prove that some of the precipitants of protid cause the precipitation of pepton: Distribute 5 ml. portions of a hot-water solution of pepton to each of several tubes, and to one add a few drops of tannic acid solution, to a second a few drops of lead acetate solution, to a third some Esbach solution. A precipitate is obtained in the first and second cases, but not in the third.[1]

The above exercises may be performed using commercial preparations of albumoses and peptons. In the United States the proprietary[2] commodities are quite free from albumoses, having been standardized for bacteriological and other special work. The German preparation known as Witte's Pepton[3] contains albumoses. The "Bacto-Pepton" of the Digestive Ferments Company and the British Savory and Moore preparation contain only traces of albumoses. If Witte's Pepton be used, the separation should begin with (2) above, the pepton being dissolved in half-saturated ammonium sulphate solution. If the other brands are used, a concentrated solution of the pepton in hot water is made, the solution is then cooled, and the study begun at (B). If desired the exercise may be repeated using the pepsin-HCl digest described on page 235.

[1] A small amount of albumose may have come through the filters, in which case some precipitation may occur.

[2] Preparations furnished by commercial houses.

[3] The word is pronounced "vitteh," if the German pronunciation is to be used. This pepton is said to be made from blood-fibrin.

Clinical Applications.— The above study is somewhat time consuming, but it is desirable that the medical student have a first-hand acquaintance with the hydrolysis products of protids. It is certain that native protids are not capable of being digested by the alimentary juices, nor are they affected by autolytic processes, that is, digestion by the tissue enzymes; for in either case there must be a chemical union of protid and enzyme to conform to the modern understanding of enzyme action.[1] In gastric analyses it is desirable to determine whether or not meat-fibers are digested; if they are recovered by the gastric tube[2] unchanged, that is, native protids rather than metaprotids, the hydrochloric acid of the stomach of the subject is not being secreted properly. Again, the introduction of albumoses into the organism leads to several characteristic conditions, such as toxemia involving fever and, locally, the formation of tissue. If large doses are given, the blood-pressure falls. It is possible that the toxemia of constipation may be due to the accumulation and absorption of albumoses.

Concerning the peptons, it may be said that the farther protid products are removed from protid, and the nearer their chemical composition approaches that of amino-acids, the lower the toxicity.

THE AMINO-ACIDS TYROSIN AND LEUCIN

The end-products of protid hydrolysis are the amino-acids. Unless the hydrolysis is carried on for some time, they do not appear and, therefore, it is impracticable to make a study of these substances directly from protid by gastric digestion. Instead, two typical amino-acids have been selected and these will be studied from preparations already made.[3]

Exercise 31.—**Tyrosin** (Fig. 106).—Take 50 mls. of the filtrate from the digest and concentrate them on the water-bath to about 10 mls. If care is exercised this process of concentration may be done

[1] Page 108.

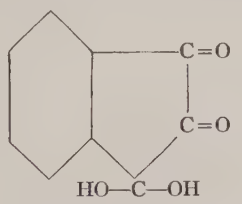
[2] Page 440.

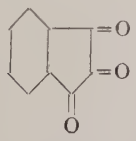
[3] They have been made from pancreatic digestion of casein (cheese). The pancreas was ground in a meat-chopper along with some of the mucous membrane of the intestine, which contains an activator of the protid-cleaving enzyme, trypsin. The material was suspended in a weak sodium carbonate solution and casein, precipitated from milk (page 411), was added; then the whole was left in a warm place for several weeks. The amino-acids, leucin and tyrosin, are readily found in this digest owing to their low solubility in cold water. Trypsin hydrolyzes 60 per cent. of casein, but if treated with pepsin first it is hydrolyzed by trypsin to 70 per cent.

over a free flame. If possible it is best to let the preparation cool and stand over night, but if time is a factor, cool the substance under the tap or in the ice-box. Filter; save the residue for tyrosin. Next add 5 volumes of water and 5 mls. of concentrated sulphuric acid. Boil. Tyrosin goes into solution, while other protid materials remain insoluble. Filter through folded paper, refiltering until a clear solution is obtained. Save the filtrate for the following Exercise; the filtrate contains tyrosin and leucin. Transfer the solution to the water-bath, add 10 mls. conc. NH_4OH, slowly, to neutrality to litmus and let the preparation cool; crystals of tyrosin[1] appear. Filter and purify by recrystallization from hot water. Transfer some to a microscope slide, cover, and examine. Combine this filtrate with that previously obtained.

There are several tests for tyrosin:

(A) *Abderhalden Ninhydrin Reaction for Amino-acids in General.* The reagent comes in the commercial package in decigram phials. Make the contents of a phial up to 10 mls. with distilled water. The solution suspected of containing amino-acid is brought to a boil, then about 1 ml. of the ninhydrin solution is added, and the boiling continued. A positive reaction is indicated by the appearance of a bluish-violet color when the solution becomes cool. The reddish tint of the violet is intensified by acid; alkali inhibits the readiness with which the color is obtained, so that a neutral reaction of the fluid tested is desirable. The formula for ninhydrin is:



It is derived from inden, a coal-tar product; hydr-inden has a ketone form 

. Through its hydrazone[2] it becomes hydrated to the form shown above, the chemical name being triketo-hydr-inden-hydrate; the commercial name is "ninhydrin." While the reagent responds to alpha-amino-acids, the reaction is given, also, by other compounds in which there are one or more terminal

[1] Fig. 106.

[2] Page 164.

carboxyls, COOH, and also one or more free α-amino-groups. Protidtemns, like peptons and peptids,[1] give a positive reaction. Substances containing amine radicles sometimes exhibit the test. On the other hand, alpha-amino-acids like prolin and oxyprolin,[2] the nitrogen of which is in an imino (NH) form, give a negative reaction.

The Abderhalden Reaction. (A) This is a serological reaction, based upon the assumption that specific enzymes are produced in body fluids when foreign protids are introduced. Abderhalden

Emil Abderhalden, Professor of Biochemistry, University of Halle a. S., Germany. Editor of several important biochemical books and compendia. (From Garrison, History of Medicine.)

applied it first to the detection of pregnancy. The blood-serum of a woman suspected of being pregnant is kept with some placenta taken from the afterbirth of another subject; this placenta has been washed free from blood and purified by dialysis from substances (amino-acids, peptons, etc.) which would give a positive reaction with ninhydrin. If the woman is pregnant, the theory is that her serum contains a protective enzyme capable of hydrolyzing the placental material, and substances like those just mentioned will arise during the digestion, which, if dialyzed from the residue of undigested placenta, boiled with ninhydrin and cooled, will give a positive test.

[1] Page 135.
[2] Pages 274 and 276.

The Abderhalden reaction has been applied to other physiological and pathological states, like cancer, dementia precox, etc. While many practitioners consider it pathognomonic in such cases, critical work has demonstrated beyond doubt that although enzymes do appear in the blood when foreign protid is introduced, there is no evidence that these enzymes are specific;[1] and unless they are, there is no basis for a differentiation between the production of blood enzymes by different types of tissue since any foreign protid will call forth enzymes of non-specific nature. The Abderhalden reaction has been applied to fluids other than blood; the urine has been used by Kiutsi[2] and by Malone,[3] but it has been shown that the method fails in critical work.

(B) *Hoffmann's*[4] *Application of Millon's*[5] *Test.*—Add a few drops of Millon's reagent[6] to not over 5 mls. of the suspected fluid in a test-tube and bring almost to the boiling-point; the appearance of a brick-red color indicates a positive reaction. For further application of the Millon test, see page 423. Folin has modified Millon's procedure so that it gives accurate quantitative results.

(C) *Piria's*[7] *Test.*—A concentrated solution of tyrosin is required. To a small amount of such solution, or to the crystals of tyrosin, add a few drops of concentrated sulphuric acid. It is best to perform the test in an evaporating dish or watch-glass, which is left on a water-bath at 100° C. for half an hour and then neutralize with calcium carbonate (barium or magnesium carbonate). Filter off the sulphate, saving the filtrate, which is treated with a few drops of ferric chlorid solution. If the reaction is positive, a violet color appears. Where small amounts of tyrosin are involved, care should be exercised that an excess amount of ferric chlorid is not used, since it masks the reaction.

(E) *Moerner's*[8] *Test; Denigès'*[9] *Test.*—These tests are practically the same, Denigès' test being conducted as follows: Place 5 mls. of

[1] See Van Slyke, D. D., Jour. Biol. Chem., vol. 23, p. 377, 1915.

[2] Japanese medical chemist; Malone, R. H., Canadian internist. See Jour. Amer. Med. Assoc., vol. 64, p. 1631, 1915.

[3] Cutter, I. S., and Morse, M. W., Jour. Amer. Med. Assoc., vol. 66, p. 599, 1916 (Cutter, I. S., Dean, College of Medicine, Northwestern University, Chicago, Ill.).

[4] Hoffman, R., German chemist of the 19th century.

[5] Millon, A. N. E., French chemist of the early 19th century.

[6] Appendix.

[7] Piria, R., French chemist, 1852.

[8] Moerner, K. A. H., German chemist, first to separate tyrosin chemically. Tyrosin was discovered in cheese by Liebig, who named the amino-acid "tyrosin" from the Greek *tyros*, cheese.

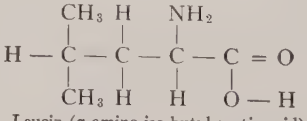
[9] Denigès, G., French chemist, 18th century.

concentrated sulphuric acid in a test-tube, add 3 drops of commercial formalin[1] and some of the suspected material. Boil and dilute with one volume of glacial acetic acid. If the test is positive, a green color develops.

Moerner's test is similar, but the reagent is made up beforehand and added to the suspected material when the test is to be performed. When the preparation is boiled a green color appears without the addition of acetic acid.

Leucin.—Exercise 32.—Separation of the amino-acid leucin: Using the combined filtrates from the previous Exercise on tyrosin,[2] isolate leucin as follows: Add ammonium hydroxid to neutralization[3] and filter, using a Buchner funnel if necessary. Now carefully acidulate the filtrate with dilute sulphuric acid, concentrate on the water-bath at 100° C., then make slightly alkaline and heat until a surface of flaky material is formed. When this concentrated filtrate is cooled over night the crystalline incrustation extends throughout the liquid. Dry the mass of crystals between filter-paper and recrystallize from 70 per cent. ethanol by heating the alcoholic solution on the water-bath; only small amounts of alcohol should be used at first, additions being made slowly. The crystals may be decolorized by means of charcoal which is added to the solution of crystals in alcohol. The solution is then heated on the water-bath, filtered while hot, and permitted to cool. Compare with the picture of leucin crystals (Fig. 97). The whiteness of these crystals prompted the discoverer, Braconnot,[4] to name them from the Greek *leuchos*, white.

Apply the tests given for tyrosin and state which are positive. The structural formula for leucin is as follows:



Leucin (α-amino-iso-butyl-acetic acid).

On account of the absence of the para-hydroxy-phenyl group, the tests (B) and (C) for tyrosin are negative. The absence of the aromatic ring prohibits condensation necessary for a positive test with formalin. Piria's test is negative. The ninhydrin reaction is positive.

[1] Page 117, note 1.

[2] Page 239.

[3] Barium hydroxid or carbonate may be used in place of ammonium hydroxid.

[4] Braconnot, H., French chemist, 1820.

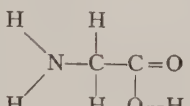
CLASSIFICATION OF THE OTHER AMINO-ACIDS[1]

Having considered the two chief amino-acids, tyrosin and leucin, we shall discuss the chemical characteristics and reactions of the other amino-acids found in protid. These are all more or less soluble in water, and may be classified as follows:

Group I. Mon-amino-monocarboxylic α-amino-acids, having one amino-group, NH_2, and one carboxyl radicle, COOH, to the molecule.[2] Besides an unbranched aliphatic chain, there may be various incorporations of elements and radicles, but in no case is there a departure from the general arrangement of amino- and carboxyl-radicles, as in tyrosin and in leucin. The general formula is $R.CHNH_2COOH$, where R may be a hydrogen atom, or a group containing an aromatic nucleus, etc.

A. Derivatives of Acetic Acid:

1. *Glycin*, glycocoll, or alpha-amino-acetic acid:



Glycin.

Glycin is the simplest of the amino-acids entering into the formation of protoplasm. It is bound into the molecule of protid in most instances, but occasionally, as in voluntary muscle of some organisms and in human urine, it occurs in a free state. It is absent in albumin, but present in globulin,[3] and thus affords a chemical means of distinguishing the two. In the "perfect food," milk, it is present in casein, but is absent in the lact-albumin; hence casein, in which the important amino-acids are represented, is a complete food. Glycin plays a major protective rôle in the body, for it unites with certain compounds, like those bearing the aromatic nucleus, benzoic acid, etc., detoxicating them and permitting their excretion from the body in a harmless state. Further reference will be made to this function of glycin.[4] Glycin is the only amino-acid which indisputably is

[1] Since 1918, when Dakin (Anglo American biochemist, contemporary) isolated an hydroxy-amino-acid (p. 246, note 2), it has been believed that perhaps other hydroxy-amino-acids occur in protids. See Schryver, S. B. and Buston, H. W., Proc. Roy. Soc., Lond., Soc. B, vol. 99, p. 476, 1926, for an hydroxyaminobutyric acid.

[2] Some amino-acids belonging here contain two amino-groups, as in cystin (page 251) but this amino-acid is virtually a condensed molecule of two separate mon-amino-acids, cystein.

[3] By treating globulin with ultraviolet light in quartz containers; globulin may be converted into albumin, but it is not known whether the glycin disappears or not.

[4] Page 739.

known to be synthesized in the body from other amino-acids, like alanin.[1] Glycin is found abundantly in tegumentary structures, derived from the ectoderm of the embryo. It was first isolated from gelatin[2] and bears the name "sweet-glue."[3] Combined with another substance, cholic acid,[4] glycin occurs in bile as glycocholic acid, one of the bile-acids. In this form, glycin serves in the digestion of fats by causing the lowering of surface tension and the consequent emulsification of the fat preparatory to digestion.[5] Glycin may be synthesized from formaldehyde and since Baly[6] has synthesized formaldehyde from CO_2 and H_2O artificially, it is possible to construct glycin from inorganic substances. Figure 96 shows the shape of

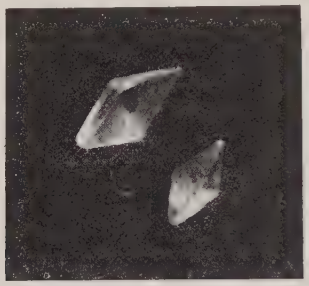


Fig. 96.—Glycin. The photograph is of crystals synthesized from non-protid substances. Magnified 4 times. (After George L. Keenan in Journal of Biological Chemistry, vol. 62, p. 165, 1924.)

glycin crystals. Glycin is optically inactive, since no carbon atom is linked with different atoms and radicles in all valencies. Glycin occurs in the urine in certain diseases, such as the toxemias of pregnancy.

2. *l-Leucin, Alpha-amino-iso-butylacetic Acid; Alpha-amino-caproic Acid.*—Leucin is mentioned again here simply for the purposes of classification. Its chief characteristics have been discussed.[7] It normally may exist in a free state in the human body, but in certain wasting diseases, such as acute yellow atrophy of the liver, phosphorous poisoning, typhoid, infantile paralysis,[8] etc., it also appears in the urine. It is of interest to the student of aviation that this amino-acid

[1] Page 247.
[2] Gelatin is obtained from the cartilages of hoofs, etc.
[3] Greek *glykys*, sweet, *kolla*, glue. Compare colloid.
[4] Page 471.
[5] Page 117.
[6] Page 143.
[7] Page 242.
[8] Poliomyelitis.

appears in the urine of persons at high altitudes. With the exception of one other amino-acid, glutamic acid,[1] leucin occurs in greatest amount by weight (10 per cent.) in casein. This shows the importance of leucin in metabolism. Leucin may be partly responsible for the acids of incomplete combustion in cases of acidosis,[2] for when it is fed to a person having diabetes mellitus, beta-hydroxy-butyric acid it considerably increased in the urine. It is probable that leucin does not contribute much to the glucose of the body, since feeding it to a dog does not result in an increase in glucose in the body. This is

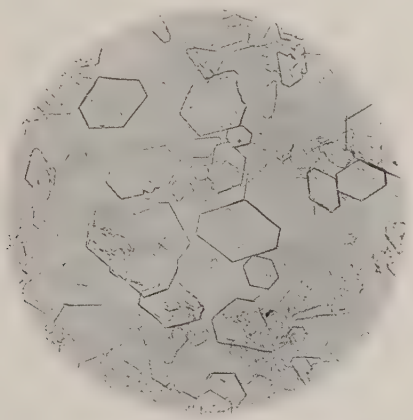
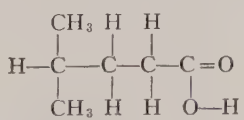


Fig. 97.—Leucin. Colorless, thin, six-sided plates and narrow, rod-like plates. Magnification as in Fig. 96. (After Keenan in Journal of Biological Chemistry.)

determined by making the dog diabetic by means of phlorhizin (Chapter XVII). This contrasts markedly with glycin, which is wholly converted into glucose under similar circumstances.[3]

The structural formula for leucin has already been given[4] as alpha-amino-iso-butyl-acetic acid. Caproïc acid, like all acids above propionic acid, may have one more hydrogen of the methyl group replaced by a methyl radicle, and thus an iso-form is produced as follows:



Iso-caproïc acid, or iso-butyl-acetic acid.

[1] Page 256.
[2] Page 81.
[3] Page 506.
[4] Page 242.

Leucin may, therefore, be considered as α-amino-iso-caproïc acid, but it is included here as a derivative of acetic acid. The closely allied iso-leucin[1] is a propionic acid derivative.

An amino-acid derived from normal caproïc acid, and called d-nor-leucin, has been described.

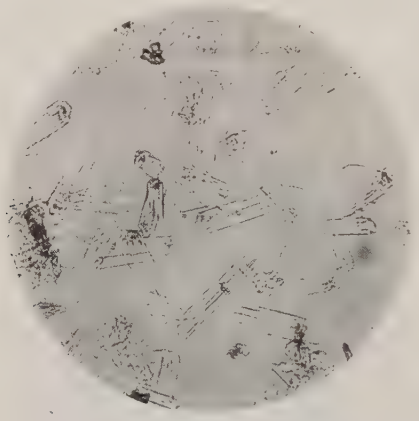
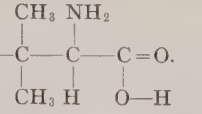


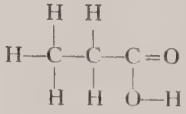
Fig. 98.—Valin. Thin, narrow, rod-shaped plates, with jagged ends and long longitudinal slits. Magnification as in Fig. 96. (After Keenan in Journal of Biological Chemistry.)

3. *d-Valin*, *α-amino-iso-valerianic acid*, or iso-propyl-alpha-amino. acetic acid:



Although a knowledge of the rôle of this amino-acid in the organism is meager, it is significant that valin occurs in casein, ranking third in percentage by weight. It also occurs in protids found in the nucleus of the cell in conspicuous quantity. Valin crystallizes with leucin and its separation is difficult. The crystals are very minute leaves.[2]

B. Derivatives of Propionic Acid.—Of the twenty amino-acids found in protids, six are constructed on the aliphatic acid, propionic acid:

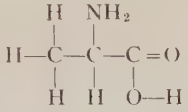


Propionic acid.

[1] Page 249.

[2] An hydroxy-valin has been described.

4. *d-Alanin, α-amino-propionic acid:*



Next to glycin, alanin is the simplest of the α-amino-acids in general structure. The organic chemist had synthesized alanin before it was found in the organism. It is convertible to glucose in the body by separation of the ammonia radicle through chemical processes of deaminization by oxidation;[1] however, there is a change

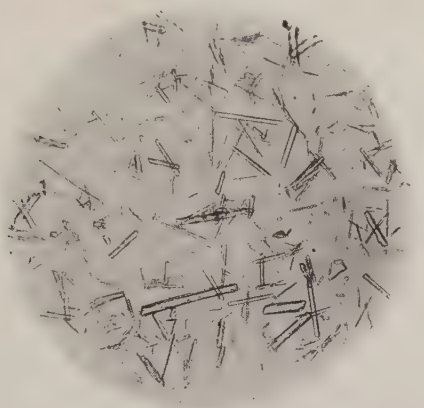
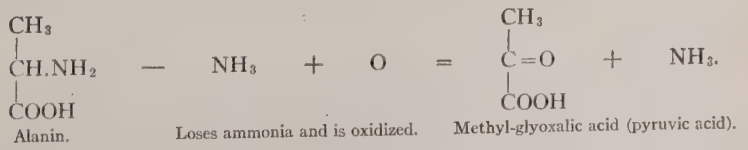


Fig. 99.—Alanin. Rods and needles as seen with microscope 10 mm. ocular and 4 mm. objective. (After Keenan in Journal Biological Chemistry.)

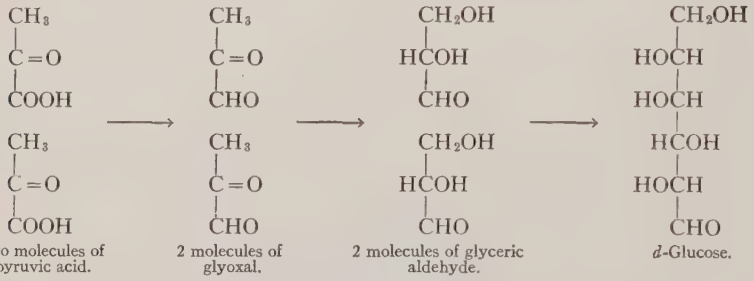
in the optical relations of the molecule. Alanin obtained in ordinary acid hydrolysis is dextrorotatory, but most naturally occurring amino-acids are levorotatory when obtained by the action of enzymes in a medium of low alkalinity, like trypsin solution used in these experiments. Glucose is dextrorotatory; hence when alanin is converted to glucose there must be some fundamental change in the molecule to cause this change in optical relation. This is accomplished by two chemical processes: (1) Deaminization by oxidation to form a ketone acid, pyruvic acid (methyl-glyoxalic acid) as follows:

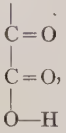


Alanin. Loses ammonia and is oxidized. Methyl-glyoxalic acid (pyruvic acid).

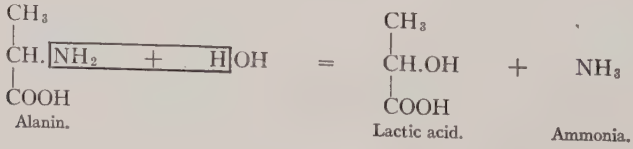
[1] Page 540, 1.

(2) Conversion of pyruvic acid to glucose, whereby two molecules of the acid are formed into the six-carbon hexose, glucose. This may be accomplished, practically, by feeding the acid to an experimental animal. The conversion was given in a reversed manner in the discussion of glucid metabolism[1] and may be represented as follows:



Hence, *l*-alanin is converted into *d*-glucose by the loss of optical symmetry when alanin is converted into the optically inactive keton-form in which the central, or alpha-carbon atom, being bound to two similar groups,  is symmetrical.[2] This conversion is of great importance in studies of metabolism in cases in which protid is converted into glucose, as in diabetes mellitus. Consequently, in prescribing diets for diabetics it is necessary to take into consideration that such a transformation of food materials may occur. Conversely, alanin may be derived from glycogen when that substance is passed through a mammalian liver by perfusion[3] with ammonium chlorid to afford the amino-portion.

Another important relation of alanin is its conversion to lactic acid as follows:



[1] Page 175.

[2] Page 178.

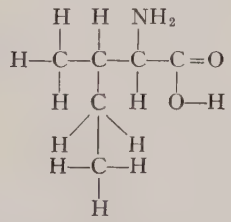
[3] Perfusion is accomplished by conducting a stream of fluid through the organ in a manner to resemble the normal course of blood. The perfusing fluid, however, contains a known concentration of some substance, the action of which in the liver is observed.

The process is that of hydrolysis. In considering the participation of alanin in sugar metabolism[1] we have shown that lactic acid can be converted into methyl-glyoxal and vice versa. Hence, the reactions given for the conversion of alanin into glucose, via keto-acid (methyl-glyoxalic acid), may be made to represent the conversion of alanin into glucose via lactic acid, by substituting the hydroxy-acid, lactic acid, for the keto-acid, pyruvic. In the body[2] this conversion is made by means of the catalyzing power of certain enzymes called *glyoxalases*, which cause a double reaction: (1) Oxidation of the hydroxy-radicle to form the keto-radicle, and (2) reduction of the carboxyl to form the aldehyde radicle. This is an application of a well-known principle in organic chemistry, the Cannizzaro reaction,[3] represented for the simple aldehyde, formaldehyde:

$$\begin{array}{l} H.CHO + O \quad = H.COOH \text{ (oxidation)} \\ \qquad\quad O \\ \qquad\quad | \\ \qquad\quad H_2 \\ H.CHO + H_2 = CH_3.OH \quad \text{(reduction)}. \end{array}$$

In the case we are considering the process is reversed (reversed Cannizzaro reaction), the carboxyl being first reduced and then the hydroxyl oxidized to the carbonyl group.[4] Two molecules of lactic acid are converted by the reversed Cannizzaro reaction to methyl-glyoxal. From this point the conversion of alanin via lactic acid to glucose may be followed in the reactions given earlier in this section.[5]

5. *d-Iso-leucin, Ethyl-methyl-alpha-amino-propionic Acid.*—This amino-acid is contained in most typical protids and has properties similar to those of leucin. Of the three possible isomers similar to iso-leucin, only the one indicated by the following formula has been found:



Iso-leucin.

[1] Page 248.

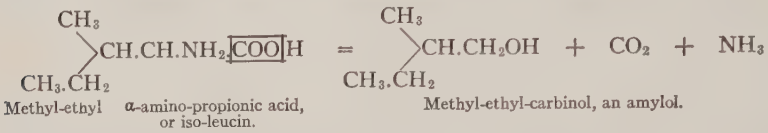
[2] Also *in vitro* (test-tube).

[3] The reaction involves a simultaneous oxidation and reduction of two molecules of an aldehyde by the addition of water.

[4] In our case the carbonyl is a keton and not an aldehyde as in formaldehyde.

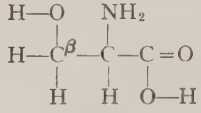
[5] Compare also the reversed action in (1) fermentation and in (2) sugar metabolism, page 176.

Iso-leucin, like leucin, is easily cleaved from the protid molecule both by acid hydrolysis and by the action of enzymes. It is converted by de-carboxylation and deaminization by yeast into amyl-alcohol, a representative of "fused oils." The reaction is as follows:



Methyl-ethyl α-amino-propionic acid, or iso-leucin. Methyl-ethyl-carbinol, an amylol.

6. *l-Serin, Beta-hydroxy-alpha-amino-propionic Acid.*—The relation of this acid to alanin is expressed in the structural formula; the methyl group of the β-carbon atom has one hydrogen replaced by an hydroxy-radicle:[1]



Serin.

Other hydroxy-acids have been identified among the hydrolysis products of protids. Serin, on reduction, becomes alanin. It crystallizes in thin leaves which, by overlapping, form an encrustation on the surface of the mass of crystals in a preparation. As a contributor to glucose serin is important since experimentally it is converted quantitatively into sugar. However, it does not usually occur in large quantities in protid; the nuclear protid and silk, the original source of the amino-acid, are exceptions.

7. *Cysteïn,*[2] *Beta-thio-alpha-amino-propionic Acid.*—This amino-acid plays a very important part in the economy of the body. It aids in destroying toxic substances[3] and it functions in oxidations.[4] Although cysteïn was discovered after cystin,[5] the latter is more complex, being virtually di-cysteïn, consisting of two molecules of the simpler amino-acid linked together. Nearly a century elapsed after the discovery of cystin before it was found that on being reduced it became a different sulphur-bearing compound of the nature of a mercaptan,[6] or organic hydrosulphid, of which ethyl-hydrosulphid

[1] Compare also the structural formulæ of cystin and cysteïn, page 251

[2] Note the diacritical marks indicating the pronunciation as "sis-te-eene." This and the following acids are levoratatory in acid solution.

[3] The sulphur becomes oxidized and, as sulphuric acid, conjugates with toxic matter.

[4] See page 99 for the participation of cysteïn in the "enzyme" glutathione.

[5] Amino-acid Number 8.

[6] See general formula for mercaptans, Appendix. From the Latin *mercurium captans*, "captor of mercury," on account of the ready action upon that element.

(mercaptan) is an example: $CH_3.CH_2.SH$. We have here an instance of the interchangeability of oxygen and sulphur,[1] for ethyl hydroxid (ethanol), $CH_3.CH_2.OH$, has oxygen in place of the sulphur atom.

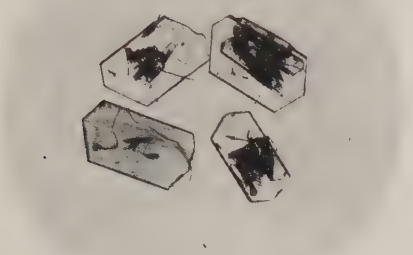


Fig. 100.—Serin. Powdered crystals of indefinite form. Magnification about 8. (After Keenan in Journal of Biological Chemistry.)

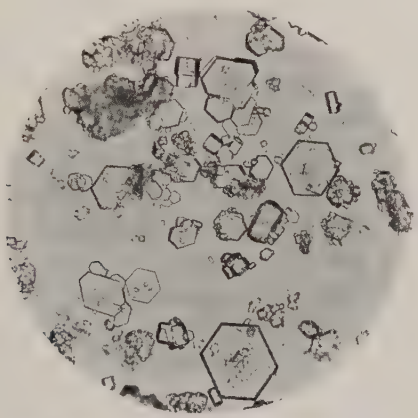
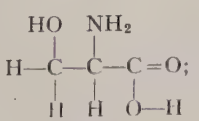
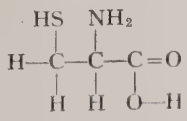


Fig. 101.—Cystin. Colorless hexagonal plates and prisms. Magnification as in Fig. 96. (After Keenan in Journal of Biological Chemistry.)

This suggests the similarity between cysteïn and serin:



Serin.

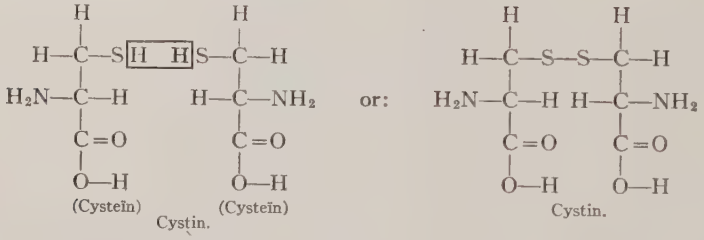


Cysteïn.

[1] Interchangeability is seen in such cases as: $2(As)(OH)_3 + 3H_2S = 6H_2O + As_2S_3$.

Cysteïn has been found in juices expressed from organs and has also been identified in the exoskeleton (hair), but the readiness with which it becomes oxidized to the di-thio compound, cystin, makes it difficult to determine the former from the latter. Recently, Sullivan[1] has described methods for distinguishing the two amino-acids.[2]

8. *l-Cystin, di-Cysteïn, Beta-dithio-alpha-amino-propionic Acid.*— This amino-acid is oxidized cysteïn, the hydrogen belonging to the sulphydryl group, —HS being removed. The formula is given to show its relation to cysteïn:



The linkage binding the two moieties is via sulphur atoms.[3]

Cystin is found in small quantities in normal urine. Pathologically, it is present in cystinuria, which may involve the excretion of a relatively large amount of the amino-acid, or even the formation of calculi of solid cystin. In cystinuria[4] there is not marked pathological manifestation, other than the appearance of unusual amounts of the amino-acid, and for this reason Garrod[5] classes it with other "inborn errors of metabolism."[6] Fed by mouth cystin does not appear as such in the urine, but is deaminized and then burned in the body in a normal manner to inorganic sulphate, H_2O and CO_2. If fed to a person with cystinuria, a small part is eliminated in the urine,[7] but the larger portion is oxidized as described. Cystin seems to be metabolized in the body in definite ways, and if cystinuria

[1] Sullivan, M. X., Chemist, U. S. Public Health Service, Washington, D. C.

[2] Page 253.

[3] Sulphur resembles oxygen and carbon in being able to bind similar atoms into chains, as in ozone, O—O, carbon compounds of the type C—C—C . . .

O

[4] Like many other physiological and pathological conditions, cystinuria bears a relation to sex, being much more common in males than in females.

[5] Garrod, A. B., Professor of Medicine, Oxford, England. See his book, Inborn Errors of Metabolism, citation, page 196.

[6] See page 268 for further examples of this group of metabolic phenomena.

[7] Other instances of substances escaping action in the body and being eliminated unchanged will be encountered, as, for example, some of the pigments (see Graphic Summary of Pigments, Chapter XV).

occurs, it is probably congenital. Cystin contributes to the formation of glucose. It likewise contributes to the formation of one of the

Fig. 102.—Howard B. Lewis, Professor of Biochemistry, University of Michigan, Ann Arbor. Investigations concerning amino-acids and their rôle in the economy of the body in normal and pathological states.

constituents of the bile, *taurin*, according to the following reactions (using cysteïn):

$$\underset{\text{Cysteïn.}}{CH_2SH \cdot CH.NH_2 \cdot COOH} \xrightarrow{\text{oxidation}} \underset{\text{Cysteïnic acid.}}{CH_2HSO_3 \cdot CH.NH_2 \cdot COOH} \xrightarrow{\text{decarboxylation}} \underset{\text{Taurin.}}{CH_2HSO_3 \cdot CH_2NH_2} + CO_2$$

Cystin is absent from some protids, of which gelatin is one; these protids are incapable of maintaining the proper supply of nitrogen when fed as foods. It is impossible to feed enough of these protids to replace the nitrogen lost in the urine.[1] Joined with the amino-acid glutamic acid[2] to form glutathione, cystin plays a most important rôle in animal functions.

Qualitative Test for Cysteïn (Sullivan[3]*).*—Principle: An hydrochinon compound is formed between cysteïn and naphthoquinon monosulphonate. The color is red or orange and does not disappear when sodium hydrosulphite, $Na_2S_2O_4$, is added.

[1] The animal cannot be brought into "nitrogenous equilibrium," which means that to replace the nitrogen lost through the urine nitrogen enough cannot be fed when administered, as, for instance, gelatin.

[2] Page 256.

[3] Sullivan, M. X., Jour. Biol. Chem., vol. 59, p. 1, 1924.

Procedure: Pipette enough amino-acid solution suspected of containing cysteïn into a test-tube to give about 7 mgs. per cent. nitrogen.[1] Add 1 drop of 0.5 per cent. solution of 1.2-naphthoquinon-4-sodium sulphonate and then 5 mls. of a 20 per cent. sodium sulphite solution made up in 0.25 normal NaOH. If cysteïn is present, a brilliant red persists. The test may be modified for detecting the presence of cystin by previously reducing the substances of the amino-acid solution; if the test is negative before reduction and positive afterward, cystin is indicated.

Quantitative Method of Folin and Looney.[2]—Principle: Cystin, alone, of all amino-acids, reacts with the uric acid reagent of Folin.[3] Procedure: To 5 mls. an of amino-acid mixture suspected of containing cystin (cysteïn), in a 100-ml. volumetric flask, add 20 mls. of saturated sodium carbonate and an equal amount of freshly prepared sodium sulphite[4] solution. Wait five minutes and then add 2 mls. of the Folin uric acid reagent. Dilute to the mark with distilled water. Compare in a colorimeter with a standard containing 3 mgs. of cystin per 100 mls. of solution in water; or, one may use a standard uric acid or glucose solution, the depth of color of which has been previously determined by colorimetric comparison with a standard cystin solution as described.

Other mon-amino-monocarboxylic acids have been described as constituents of protid, for example, a sulphur-bearing amino-acid besides cystin.[5] No acid corresponding to the butyric acid with two possible isomeres, or a pentane homologue has been described, and it is improbable that such acids will be discovered in protid, since extensive and very exact work has already been done in the analysis of animal and plant tissues, and in many instances the analyses do not permit the presence of more amino-acids judged by the total nitrogen of the protid. This completeness of our knowledge is principally due to the method of analysis devised by Dakin.[6]

[1] A preliminary nitrogen determination may be made by the Folin method, Chapter XV.

[2] Looney, J. M. See title page.

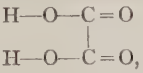
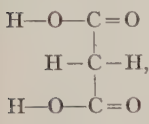
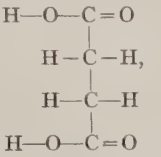
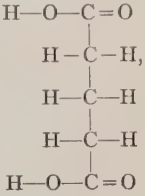
[3] It is actually cysteïn that reacts with the uric acid reagent, but cystin is reduced in the process.

[4] $Na_2SO_3.7H_2O$ saturated solution, 30 gs. per 100 mls. solution in water.

[5] $CH_2.SH(CH_2)_2CHNH_2.COOH$. See Mueller, J. H., Jour. Biol. Chem., vol. 58, p. 373, 1923. This substance has been identified by Eddy (page 24) as "bios," but the point remains unsettled. See Tanner, F. W. (Illinois), Chem. Revs., vol. 1, 397, 1925.

[6] Dakin, H. D. Anglo-American chemist, an editor of the Jour. Biol. Chem. and an intensive student of biochemical substances and processes.

Group II. Mon-amino-di-carboxylic Acids.—Alpha-amino-acids having two carboxyl groups, hence frequently called the acidic amino-acids. Two of many possible compounds of this series are present in the hydrolysis products of protids:

Succinic acid derivative: Succinic acid is a member of the di-basic acid series which begins with oxalic,  , then malonic,  , then succinic,  , then glutaric,  , etc. Glutaric is the second compound of this series which contributes to the formation of amino-acids found in protids.

9. *l-Aspartic Acid, α-amino-succinic Acid.*—The name of this amino-acid suggests asparagus, and such an association does exist.

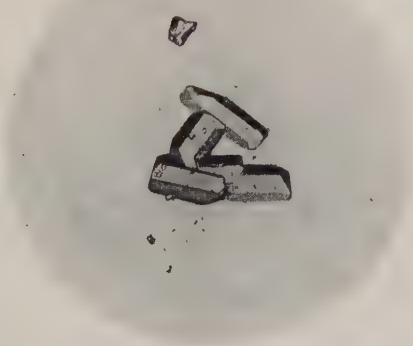


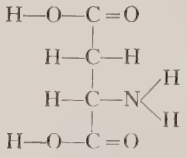
Fig. 103.—Aspartic acid. Irregular fragments of crystals of high refractive indices. Magnification as in Fig. 96. (After Keenan in Journal of Biological Chemistry.)

Aspartic acid is present in many plant substances[1] in fairly generous amounts, and hence its presence in asparagus is not conspicuous. However, the mon-amid, asparagin, which was the first source and still is the chief source of aspartic acid, forms a large part of the dry substance of asparagus; this accounts for the derivation of the term. Asparagin[2] was first derived from asparagus at the beginning of the

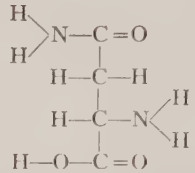
[1] For example, in legumes it may make up about 5 per cent. of the total amino-acid content.

[2] This mon-amid is also present in the seedlings of plants, sometimes to the extent of 5 per cent.

nineteenth century in France. Fifty years later aspartic acid was recognized as a constituent of most protids, those occurring in the nucleus of the cell being exceptions. Aspartic acid is the alpha-amino compound of succinic acid, while asparagin is the acid amid[1] of that acid:



Aspartic acid (α-amino-succinic acid.)



Asparagin (α-amino-succinic-acid-amid).

Aspartic acid enters the formation of glucose with but three of its carbon atoms, which probably means that it is converted into glucose in a manner similar to that already described for alanin;[2] in fact, alanin has been obtained from the acid hydrolysis of aspartic acid. Aspartic acid, by decarboxylation, becomes a beta-amino-acid:

$$HOOC.CHNH_2.CH_2.COOH \longrightarrow CH_2.NH_2.CH_2.COOH.$$

Fig. 104.—Glutamic acid. Magnification as in Fig. 96. (After Keenan in Journal of Biological Chemistry.)

10. *d-Glutamic*[3] *Acid, Alpha-amino-glutaric Acid.*—This amino-acid is one of the most widely distributed of the series and occurs in large amounts in many protids. In wheat, for example, it forms

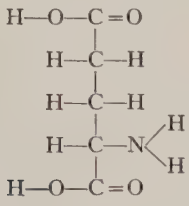
[1] See Appendix for general structure of acid amids. An acid amid is an organic acid which has its hydroxyl of the carboxyl replaced by an amino-radicle, NH_2, represented as follows: R—C(=O)—O—H (carboxyl); R—C(=O)—H—N—H (amid).

[2] Page 247.

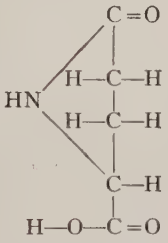
[3] Glutaminic, as sometimes spelled. An hydroxy-acid, β-hydroxy-glutamic acid has been described by Dakin in the Biochemical Journal for 1918, vol. 12, p. 290.

nearly half the total amino-acid content, and in the casein of cheese, nearly one-quarter. The wide-spread use of wheat as human and animal food has stimulated interest in the metabolism of glutamic acid to such an extent that we now know many of its characteristics.[1] A compound of glutamic acid and cystin, glutathione, has been mentioned and will be considered in detail later.[2] It is interesting in this connection that Abel (page 511) has found sulphur associated with insulin; sulphur occurs in glutathione, as a part of cystin.

Glutamic acid, chemically, is a derivative of glutaric acid, the amino-group of this diamino-acid being attached to the gamma carbon atom. A lactam may occur analogous to a lactone and glutamic acid exhibits such a formation:



Glutamic acid.



Lactam of glutamic acid.

In burns, in liver atrophy, and in other conditions in which there is extensive alteration of protids, the associated toxicity has been attributed to the end-products of protid metabolism, but amino-acids are not in themselves toxic. However, since lactams are toxic, it may be the formation of such compounds during pathological states that is responsible for the toxicity, for compounds bearing the aromatic ring are generally incompatible with the usual form of metabolism of the animal body. The ring is split only rarely; ordinarily it is conjugated with some other substance[3] to render it non-toxic. The toxicity in such cases must arise from glutamic acid, for it is the only amino-acid in protids capable of forming lactams. The amin of glutamic acid, glutamin, corresponding to asparagin appears in the urine of man after it has been conjugated with phenyl-acetic acid, an aromatic acid. It also appears to be synthesized in man living on a plentiful glucid, but low protid diet.

Examination of the formula for the lactam of glutamic acid reveals its relation to a ring formation which is characteristic of the red coloring-matter of the blood, hemoglobin, and of the green

[1] Page 310.

[2] Pages 343 and 344.

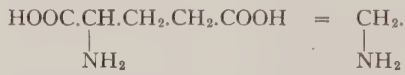
[3] Compare glycin and cystin, page 252; Fig. 53.

coloring-matter of leaves, chlorophyl. These substances have similar respiratory functions:

Lactam of glutamic acid (pyrrolidin carboxylic acid).

Pyrrolidin.

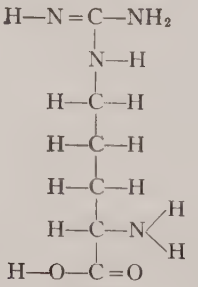
Both chlorophyl and hemoglobin have four of these rings and glutamic acid may be a source of hemoglobin. Glutamic acid by decarboxylation becomes a γ-amino-acid:



compare aspartic acid, which is converted similarly into a β-amino-acid.

Group III.—Di-amino-mono-carboxylic Acids.—These α-amino-acids bear two amino-groups and a single carboxyl radicle, hence they are spoken of as the basic amino-acids. They form with histidin[1] a small group of amino-acids sharply set off from all others by their basic character. Histidin has a heterocyclic ring. It contains two amino-groups. All three of these amino-acids can be separated from the other protid cleavage products by precipitating the diamino-acids with phosphotungstic acid. The two diamino-acids of the aliphatic series are as follows:

11. *d-Arginin, α-amino-δ-quanidin-valerianic Acid.*—The formula is as follows:



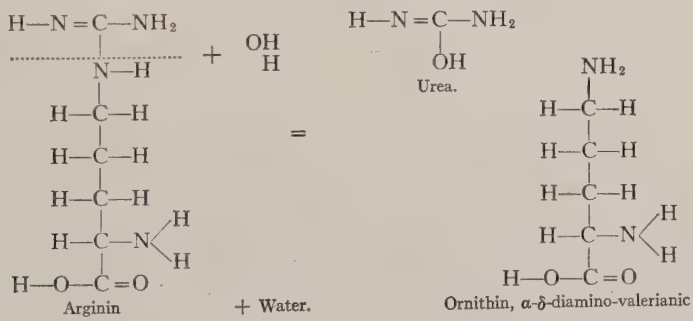
Arginin.

The strong basic property is due to the presence of the two amino-groups. This property is noted in certain protids in which diamino-acids form a large part of the molecule. This is the case in the nuclear protids of the spermatozoa. Arginin forms nearly 90 per cent. of the

[1] See amino-acid number 15, page 272.

total amino-acid content of the protid salmin from the spermatozoa of the salmon. Strongly acid or basic substances do not exist freely in the body, and we should expect to find the basicity neutralized by an acid substance. This is true for salmin in which the nucleic acid of the nucleoprotid is linked with the strongly basic protid.[1] Arginin is quantitatively precipitated by the organic acid 2-4-dinitro-7-naphtholsulphonic acid. Arginin is not amphoteric in the sense that the acidic and basic portions (carboxyl and amino groups, respectively) are balanced, for there is a preponderance of the basic amino-part. Hopkins[2] finds that arginin is responsible for one portion of the uric acid excretion in the urine.

The epsilon carbon or arginin is linked with three nitrogenous groups, two of which are in an imino, NH, form. This configuration resembles closely that for guanidin: $H_2N-C(=N-H)-NH_2$. This radicle may be cleaved from the molecule of arginin by hydrolysis, but the guanidin residue ceases to be guanidin and becomes urea:



Urea and ornithin result. Ornithin is alpha-delta-diamino-valerianic acid; valerianic acid is $CH_3.CH_2.CH_2.CH_2.COOH$. Guanidin is encountered in a methylated[3] condition linked with acetic acid as creatin,[4] but no association has ever been discovered between arginin and creatinin adequate to explain the facts. Guanidin has been reported from the blood of children suffering from tetany[5] and in the urine of experimental animals from which the parathyroid

[1] Page 306.

[2] Page 221.

[3] The methyl group, CH_3, is added to the molecule.

[4] Page 364.

[5] A disease due to parathyroid deficiency. See page 661. (Not tetanus or lockjaw, the cause of which is the toxin secreted by the bacterium Clostridium tetani.) For the nomenclature of bacteria and for frequent courtesies in the course of the preparation of this book the author's thanks are due Professor Rosenberger, Head of the Department of Hygiene and Preventive Medicine, Jefferson Medical College.

glands had been removed.[1] When guanidin or its salts are introduced into the blood-stream of an animal, hypoglycemia,[2] loss of calcium from 5 to as low as 2 mgs. per 100 mls. of blood, and acidosis ensue. When the parathyroids are removed, guanidin disappears from the muscle. Parathyroids seem to control the metabolism of guanidin, and since it occurs in only one known amino-acid, it is quite probable that the metabolism of this amino-acid is under the control of the parathyroids. There is also some connection between the metabolism of calcium and the presence of guanidin. Deprivation of the parathyroids results in a change in calcium metabolism. Apparently an intimate relation exists between these different factors. It has been suggested by Paton[3] that the parathyroids regulate the concentration of guanidin and in turn the muscle tonus. It is known that administration of guanidin causes hypertonicity of muscle (page 364).

Ornithin is affiliated with poisonous substances of similar chemical composition, such as the so-called "ptomaïns."[4] One of these, putrescin, is derivable from ornithin as follows:

$$\underset{\text{Ornithin.}}{NH_2.CH_2.CH_2.CH_2.CH.NH_2.COOH} \xrightarrow[-CO_2]{\text{decarboxylation}} \underset{\text{Putrescin.}}{NH_2.CH_2.CH_2.CH_2.CH_2.NH_2.} + CO_2$$

This is a type of decarboxylation, which is a property of bacteria, but is seldom encountered in human or animal biochemistry.[5] The feces of patients with certain diseases like cholera contain putrescin and cadaverin and other decarboxylated substances; also in cystinuria these substances have been discovered in the excretions, and it has been shown by Berlin biochemists that decarboxylations occur in some tissues. Cadaverin is derived from the amino-acid about to be discussed, lysin.

Arginin is the only amino-acid capable of producing urea directly. However, all amino-acids are capable of being de-aminized, the ammonia becoming condensed with cyanic acid to form urea.[6]

[1] Page 661. This condition is known as tetany thyreopriva, a designation belonging to an earlier stage of medical history, when the thyroid and parathyroid were not distinguished.

[2] Lowered blood-sugar concentration.

[3] D. Noël Paton, British biochemist and physiologist.

[4] The rôle of such toxins as the cause of death in "ptomaïn poisoning" is slight. Death from eating spoiled meat, etc., is due to infection from certain strains of bacteria, like Clostridium botulinum, producing "botulism" (Latin, *botulis*, sausage).

[5] It will be recalled that Leathes utilized this principle of decarboxylation in explaining the change of an odd-carbon fat to an even one, which is capable of being metabolized (page 204).

[6] Page 701.

We have previously called attention to the occurrence of arginin in nucleo-protids, which occur as "chromatin" in the nucleus. Albumin and globulin of the blood show a variation in the content of amino-acids; the figures are given on page 320 for the percentage weights of the various amino-acids in serum albumin and serum globulin.

The average for the amino-acids of the diamino group (arginin, lysin, and histidin), compared with that for the remainder of the amino-acids, is given:

Average of the concentration percentage of diamino-acids:

	Grams, per cent.
Albumin	6.6
Globulin	4.2

Average of all the other amino-acids:

	Grams, per cent.
Albumin	3.5
Globulin	3.1

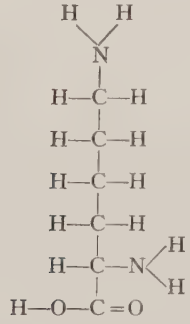
In other words, there is a preponderance of diamino-acids in serum protid; the concentration of diamino-acids is greater in albumins than in globulins.[1] These figures are to be compared to the amino-acid content of simple protids which occur in the nucleus (page 306).

12. *"Active" Lysin, Alpha-epsilon-diamino-caproïc Acid.*[2]—This, like arginin, is widely distributed. Nevertheless, its absence from certain common protids like the zeïn of corn (maize) is conspicuous, and has been responsible for some of the most important studies concerning the metabolism of foods. These studies have shown that the conception, initiated by von Liebig in the earlier days of biochemistry, that the whole of the protid molecule is used as such as food, is incorrect, and that the unit is the amino-acid of which the protid is made rather than the molecule of the protid. Zeïn is incapable of furnishing enough nitrogen to bring the organism to which it is fed into nitrogenous equlibrium, not because there is insufficient nitrogen in the molecule of the protid, zeïn, but because the nitrogen of one unit, lysin, does not occur in the molecule. It is as if an archway were being built and there were various sorts of stones available, but no keystone; the stones as a whole (the molecule) are inadequate to form the arch, for the keystone (lysin) is not among them.

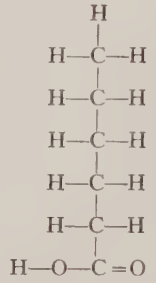
[1] Page 320.

[2] Anhydroxy-lysin has been described by Schryver and collaborators. See Proc. Roy. Soc. London Series B., vol. 98, page 58, 1925.

The body can be maintained for a time, at least, on a diet lacking in lysin, but growth is impossible on such a diet. Later we shall discuss the rôle of amino-acids in maintenance and growth.[1] The formula for lysin is as follows; it is derived from normal caproïc acid,[2] leucin being derived from an iso-caproïc acid:[3]



Lysin (α-ε-alpha-amino-caproïc acid).



Normal caproïc acid.

Reference may be made to the previous discussion of the conversion of arginin into the amin, putrescin, by decarboxylation. In separating the three amino-acids known as "hexone bases"[4] (arginin, lysin, and histidin) from one another, arginin and histidin are precipitated by adding silver sulphate solution to their solution, thus forming silver arginin and silver histidin. These, in turn, are separated into histidin hydrochlorid, $C_6H_9N_3O_2.2HCl$, and arginin nitrate, $C_6H_{14}N_4O_2.HNO_3.\frac{1}{2}H_2O$. Lysin is precipitated by means of phosphotungstic acid after the silver compounds have been removed and is converted into the picrate $C_6N_{14}N_2O_2.C_6H_2(NO_2)_3OH$. The pure compound of amino-acid may then be obtained by removing the HCl, HNO_3, or picric acid from these substances. Recently Kossel has invented a precipitant for these acids which is known as Acid Naphthol Yellow-S.

[1] Page 549.

[2] See page 245 concerning another amino-acid derived from caproic acid.

[3] Page 245.

[4] The term "hexone bases" was applied by the German biochemist Kossel to these three amino-acids, but since the exact chemical constitution of histidin has been discovered together with the capacity of the acids to enter into the formation of glucose, the term has become meaningless. The term "hexone" referred to the fact that there are 6 carbon atoms in each of the acids and it was supposed that they contributed to the formation of glucose (a hexose). The 6 carbons occur as follows:

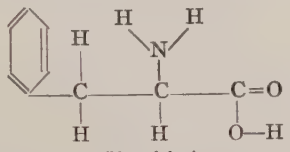
$C_6H_{14}N_4O_2$ ..... arginin
$C_6H_{14}N_2O_2$ ..... lysin
$C_6H_9N_3O_2$ ..... histidin

Lysin differs from all other amino-acids in that it does not participate in the formation of glucose. This is because lysin becomes oxidized to the dicarboxylic acid, glutaric acid,[1] which, being a 5-carbon chain, does not form a hexose, either directly or indirectly. Lysin does not form acids of incomplete combustion, like β-hydroxybutyric acid, which occur in the blood and urine during acidosis.[2] By correlating these facts with those given in the discussion of histidin it will be evident why the term "hexone bases" is undesirable.

Group IV.—Homocyclic Amino-acids.—This group consists of two acids, each having an aromatic nucleus conjugated with propionic acid.

Benzene derivative:

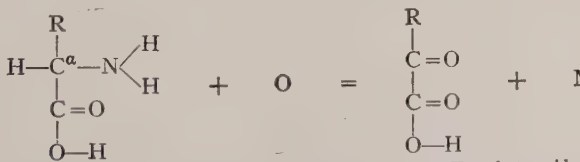
13. *l-Phenylalanin, Beta-phenyl-alpha-amino-propionic Acid.*—The formula is:



Phenylalanin.

The place of attachment of the propionic acid radicle is not fixed, since the ring is symmetrical. Compare the next amino-acid, tyrosin, where the point of attachment is fixed, because there is an hydroxy-radicle in para position. Phenylalanin is present in most protids except perhaps casein of milk. Nevertheless milk is practically a perfect food-protid, since lactalbumin does contain phenylalanin.

The metabolism of the acids of this series[3] involves an additional factor: the aromatic nucleus. All alpha-amino-acids become oxidized on their alpha carbon atom, the atom bearing the amino-group, whether metabolism is normal or not. Oxidation leads to deaminization of the amino-acid according to the following scheme, in which R is any radicle like methyl, etc.:



An amino-acid. Deaminization by oxidation. Keto-fatty-acid.

[1] Page 255.

[2] Page 81.

[3] Lewis (p. 253) has shown that the metabolism of the two members of this group is different. Phenylalanin is converted into an orthodiphenol, at least in part, but none is found after administering tyrosin.

An alpha-keton acid arises and ammonia is split off. It will be observed that since R may represent an aromatic nucleus, it remains attached to the compound after the ammonia has been cleaved from

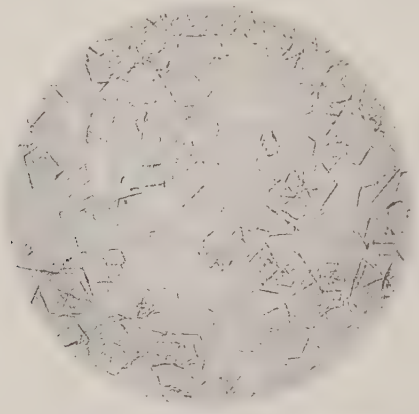
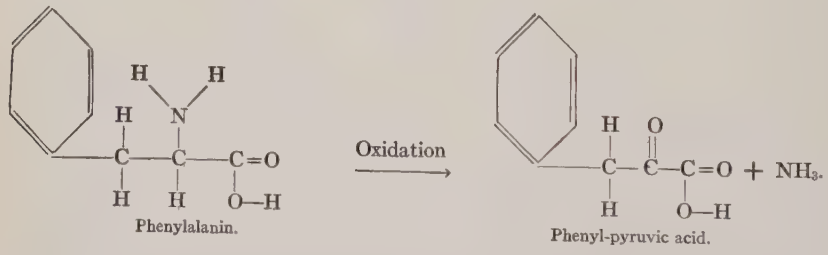


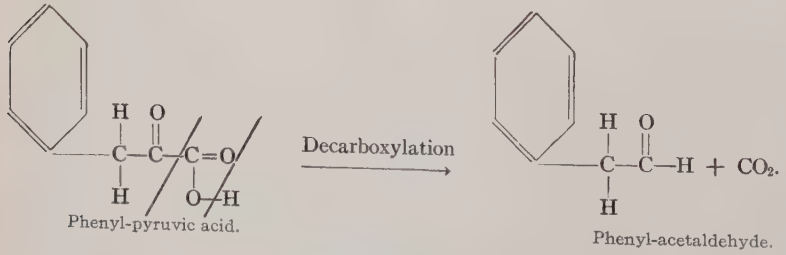
Fig. 105.—Phenylalanin. Hexagonal plates. Magnification as in Fig. 96. (After Keenan in Journal of Biological Chemistry.)

the molecule. Therefore the deaminization of phenylalanin by oxidation leads to the formation of the keton acid, phenyl-pyruvic acid:



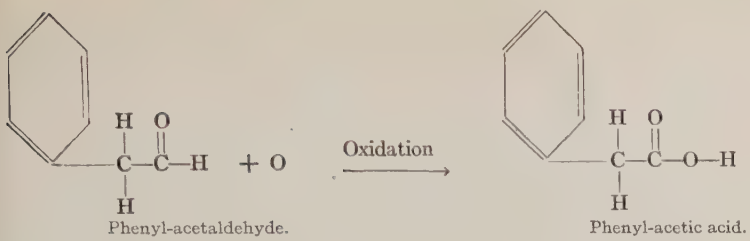
Phenylalanin. Phenyl-pyruvic acid.

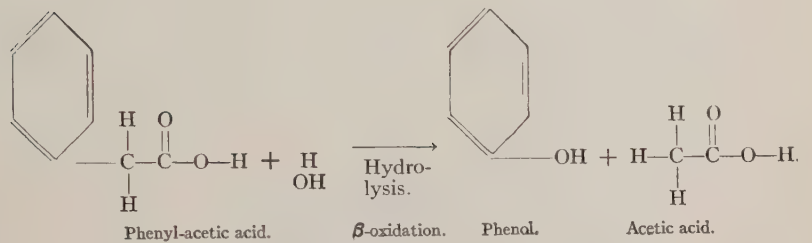
Thenceforward oxidation occurs as beta-oxidation;[1] that is, in the case of phenylalanin, which has been converted into phenyl-pyruvic acid, the following reactions occur:



Phenyl-pyruvic acid. Phenyl-acetaldehyde.

[1] Compare the oxidation of fats, page 202.

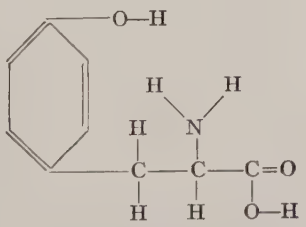




Phenol is excreted, when conjugated with sulphuric acid, as phenylsulphuric acid, one of the "ethereal sulphates" in normal human urine.[1] It is clear that the benzene ring remains attached to the fatty acid as the chain of carbon atoms becomes reduced. This is similar to the method whereby the fats are metabolized, that is, by beta-oxidation.[2] Lactic acid may arise from phenylalanin in a manner similar to that by which alanin is converted into ordinary aliphatic lactic acid.[3]

Phenol derivative:

14. *l-Tyrosin, Para-hydroxy-phenyl-alpha-amino-propionic Acid.* The formula for this acid differs from that of phenylalanin in that an hydroxyl is incorporated in the position para to the aliphatic radicle:



[1] Page 795.

[2] The process is shown above as one of hydrolysis, for the reason that it is easier to present the matter in this manner; the process is actually one of oxidation.

[3] Page 248. Read note 3, page 263 relative to the passage of phenylalanin through a di-phenol stage in its oxidation.

In tyrosin[1] we have a very important amino-acid and one which is worthy of detailed study. It occurs in many protids, but is absent from gelatin derived from hoofs, etc. It is not alone the presence of the aromatic nucleus which is important, for, as has already been pointed out, animals fed gelatin cannot be brought into nitrogenous equilibrium, although the benzene ring, which is contributed by phenyl-alanin, is present. If a protid is to be used by the body, it must contain a definite radicle. As an example of this, we may cite the fact that one cannot cause iodin to enter the molecule of phenylalanin directly, but tyrosin, having a definite radicle, may be iodized, as in the artificial formation of one of the constituents of the thyroid

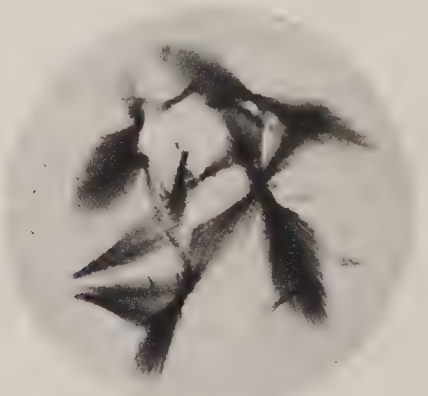


Fig. 106.—Tyrosin. Needles and rods are colorless. Magnification 80. (After Keenan in Journal of Biological Chemistry.)

gland; the iodized tyrosin is known chemically as gorgoïc acid, 3-5 di-iodo-tyrosin.[2] Here the difference involves simply the addition of an hydroxyl. The structure of tyrosin was determined during studies of the chemistry of the thyroid gland.

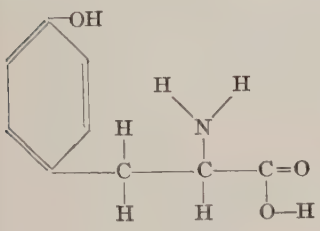
Tyrosin is closely concerned with the production of certain pigments. If an insect be kept in an atmosphere of hydrogen or nitrogen as it emerges from its chrysalis, pigment does not form, but on exposing it to the oxygen of the air color appears. Detailed studies of the phenomena of pigment formation have been made by Gortner[3] and others, and it has been shown that an enzyme, *tyrosinase*, isp resent to catalyze the reaction. In certain forms of cancer, like melanotic

[1] See also page 238.

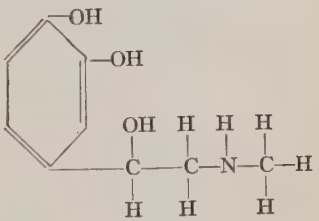
[2] See page 271.

[3] Gortner, R. A., Chief of Chemical Division, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

sarcomas,[1] tyrosinase occurs, which oxidizes the tyrosin to the "melanins," a group name for condensation products not only of tyrosin, but of others of the aromatic amino-acids.[2] Tyrosin plays another rôle in that it acts as a mother-substance of an important chemical regulator of the body—epinephrin or adrenalin[3]—secreted by the glands lying over the kidney in man. It is a substance which, when injected into the body causes a constriction of the blood-vessels and hence a rise in the pressure of the blood. Although as yet we have been unable to derive epinephrin from tyrosin, the similarity in their constitution is shown by their formulæ:

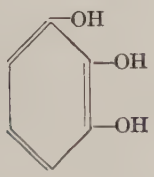


Tyrosin.

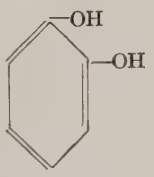


Epinephrin.

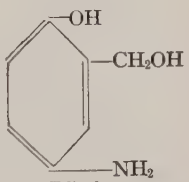
The chemical relationships of epinephrin are with the catechols, the group to which some of the developing reagents of photography belong:



"Pyro," pyrogallol.



Catechol.



Edinol.

These substances share with epinephrin the characteristics of turning dark when exposed to oxygen. The same property is encountered in urine in cases of alkaptonuria,[4] the tyrosin derivative, alkapton, or homogentisic[5] acid, being present. This is a second

[1] Greek *melas*, dark, and *sarx*, flesh; tumors composed of embryonic cells and pigmented with black or brown.

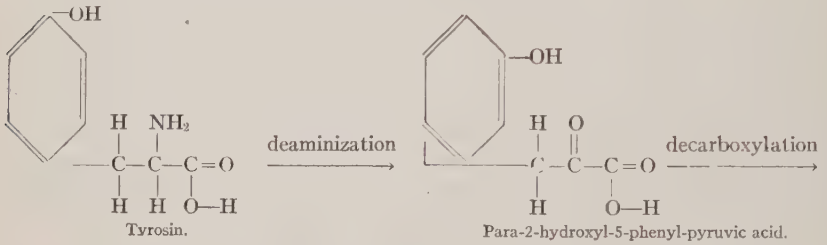
[2] There is probably an adaptive mechanism whereby melanin appears in structures exposed to light, for the aromatic amino-acids protect the underlying structures from the harmful effects of short wave-length light.

[3] Greek *epi*, upon, and *nephros*, kidney; Latin *ad*, toward, and *ren*, kidney. Compare the betaïn formula, page 283.

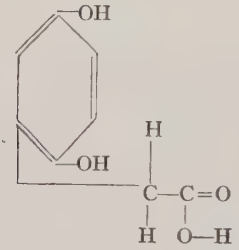
[4] From alkali and the Greek *'apto*, to fasten; signifying the necessity of alkaline state.

[5] From the Greek *'omos* (homos), like, and *gentisic*, the acid akin to homogentisic acid; gentisic acid is $C_6H_3(OH)_2COOH$, so named from its source in the gentian, a plant.

instance of what has been termed an "inborn error of metabolism,"[1] that is, an altered metabolism without special pathological symptoms involving detrimental conditions. Homogentisic acid is theoretically derived from tyrosin as follows:



Tyrosin. Para-2-hydroxyl-5-phenyl-pyruvic acid.



1-4-Hydroxy-5-phenyl-acetic acid (homogentisic acid).

It is evident that the attachment of the hydroxyls of homogentisic acid to the aromatic nucleus is different from that of tyrosin, but shifts like these are encountered in chemical changes; from analogies with other shifts we know that the aliphatic (acetic acid) group has shifted, and not the hydroxyls.

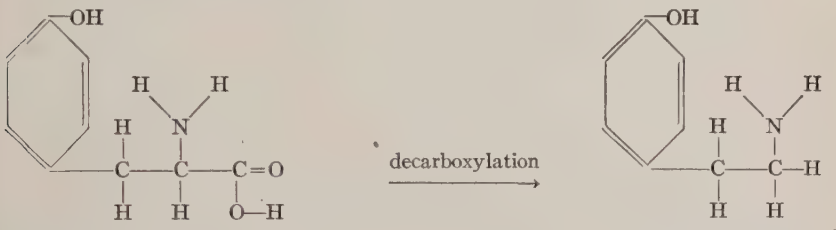
Alkaptonuria is a rare condition, apparently hereditary. It probably belongs with such irregularities as albinism. The condition is known to be present even when some of the tyrosin or other aromatic amino-acid is metabolized in a normal manner, and the older idea that bacterial decomposition is the cause of the appearance of the acid is no longer held. Homogentisic acid probably is not an intermediate product in normal tyrosin metabolism. If it is brought into contact with the serum of a non-alkaptonuric subject it is destroyed. Pathological changes do not occur to any extent in alkaptonuria, but there may be accessory difficulties, like the deposition of homogentisic acid in the joints, giving rise to an arthritis.[2]

[1] Pages 252 and 268.

[2] Greek *arthron*, joint, and *itis*, a disease of (the word for disease is *nosos* and *-itis* is the feminine plural, to agree with the noun denoting what part of the body the disease affects; the word *nosos* is now left unwritten, but understood).

Tyrosin appears in the urine of subjects with atrophy of the liver, either acute yellow atrophy due to the self digestion of the liver tissue by the liver-enzymes, or a similar condition due to phosphorus poisoning. Over 1.5 gms. of tyrosin per twenty-four hours may be recovered in some cases of acute yellow atrophy, but it does not appear in any such quantities in cases induced experimentally by feeding poisonous phosphorus.[1]

Tyrosin is acted upon by bacteria[2] in the intestine and become converted by decarboxylation into substances of poisonous nature; thus it becomes an amin, *tyramin*, having the characteristics of causing a rise in blood-pressure. This may be the agent which induces the increased blood-pressure in intestinal obstruction, like chronic constipation, appendicitis, adhesions, etc.:



Tyrosin, *p*-hydroxyl-phenyl-α-amino-propionic acid.

Tyramin, *p*-hydroxy-phenyl-ethyl-amin.

This amin is a natural constituent of the pharmacological substance, ergot,[3] which has a counterpart in histamin, derived from histidin. The use of ergot in obstetrics does not involve the presence of tyramin, but histamin enters actively into the physiological use of ergot. It[4] causes a reversal of the action of tyramin on blood-pressure, that is, it lowers blood-pressure. It also exerts a strong action on the musculature of the uterus, causing it to contract.[5] Like the amins

[1] This condition arose and still arises in countries in which matches are made of yellow phosphorus. As a consequence of its high toxicity, yellow phosphorus is prohibited in the manufacture of matches in most countries. Red, non-poisonous phosphorus is used in coating the boxes of "safety" matches.

[2] Certain strains affect this amino-acid, while others produce an amin from histidin. The reason why bacteria form amins is given by Hanke and Koessler (University of Chicago), who find that it is a protective mechanism resorted to when the medium in which they live is unusually acid. These basic substances, like tyramin, histamin, etc., act as buffers (page 73). See Jour. Biol. Chem., vol. 59, 855, 1924.

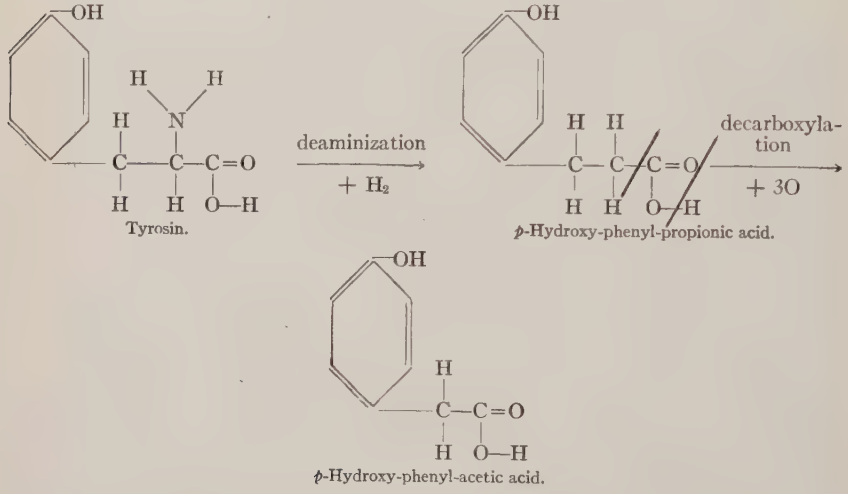
[3] Ergot is a fungus parasite of grains, especially of rye, from which ergot is obtained for commercial purposes. The resting stage of the reproductive organs affords the maximum amount of the drug. It is used in obstetrics to cause stoppage of the flow of blood after the child is born. The action is upon the smooth musculature of the uterus, which is caused to contract.

[4] For the comparative action of epinephrin, tyramin, and histamin see page 274.

[5] Page 274.

of the other amino-acids having an aromatic nucleus, tyramin affects the body in a manner similar to that of the sympathetic system, and the amins are called the sympathetico-mimetic substances.

Finally, if tyrosin be deaminized, it becomes first *p*-hydroxy-phenyl-propionic acid, which, in its turn, is converted into the acetic acid form by decarboxylation and oxidation.

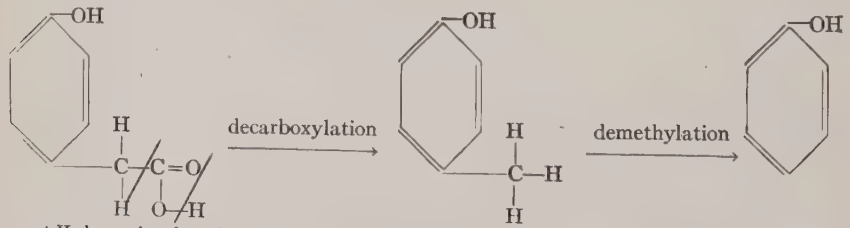


Tyrosin.

p-Hydroxy-phenyl-propionic acid.

p-Hydroxy-phenyl-acetic acid.

From the acetic form it is converted by decarboxylation into methyl-phenol, or cresol (para-cresol), and by oxidation into phenol, being excreted as such in the urine, or as a conjugated sulphuric acid, phenyl-sulphuric acid;[1] or conjugated as a glucuronate with glucuronic acid:[2]



p-Hydroxy-phenyl-acetic acid.

p-Cresol.

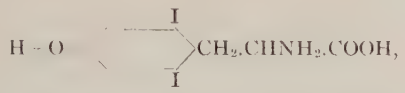
Phenol.

The liver has been considered the chief organ for detoxications, but it is possible that the intestinal epithelium plays its part in the process.

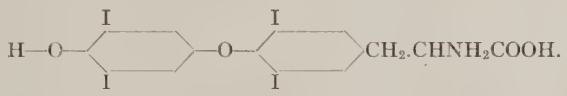
[1] Page 280. These substances make up the so-called "ethereal sulphates" of the urine.

[2] Page 281.

Thyroxin.— According to Harington and Barger,[1] thyroxin (page 99) is a tyrosin derivative, being constructed something like 3-5-di-



iodo-tyrosin, inasmuch as it is an hydroxy-benzene radical attached to an alaphatic amino-acid and containing for each, aromatic radicle, of which there are two, a molecule of iodin:



Folin and Looney's Quantitative Method for Tyrosin.—Principle: Tryptophan, if present, is separated[2] from tyrosin. This is accomplished by mercuric sulphate which precipitates tryptophan. The compound is centrifuged and the supernatant liquid is used for tyrosin analyses.

Procedure: Using a Folin pipette[3] transfer a given amount of the amino-acid mixture known to contain tyrosin into a 15-ml. centrifuge tube. Add 2 mls. of the Hopkins-Cole mercuric sulphate-sulphuric acid reagent.[4] Add enough 5 per cent. H_2SO_4 solution to make a total volume of 10 mls. in the tube. Stopper and invert the tube, then shake it vigorously in order that the contents are thoroughly mixed. Let stand two hours. Centrifuge two minutes at about 1800 R. P. M. Decant the clear supernatant liquid into a test-tube. Tyrosin determination: Pipette 5 mls. of the supernatant liquid into a 100-ml. volumetric flask. To a second, similar flask, add 1 ml. of a standard tyrosin solution made up in 5 per cent. H_2SO_4, each ml. of which contains 1 mg. of tyrosin. Add also to this flask 1 ml. of the mercuric sulphate—H_2SO_4 solution and 3 mls. of 5 per cent. sulphuric acid. To both flasks add 30 mls. of distilled water, 20 mls. saturated sodium carbonate and 4 mls. of 5 per cent. sodium cyanid solution, and, finally, to each flask add 2 mls. of the special phenol reagent.[5] Mix, let stand half an hour, make up to the mark and compare in a colorimeter, standard at 20 mms.

[1] Harington, C. R. and Barger, A., Paper presented to the Biochemical Society, (London, Eng.) December, 1926. See page 141.

[2] And may be determined later; the method is given under tryptophan.

[3] Fig. 37, page 78.

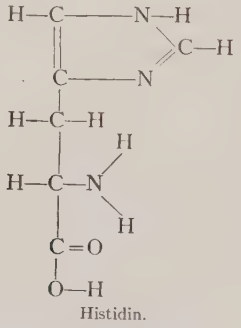
[4] Appendix. See Cole's Practical Physiological Chemistry, p. 89, C (i), edition 1919.

[5] Appendix. The addition of a small amount of lithium sulphate will prevent the formation of a precipitate.

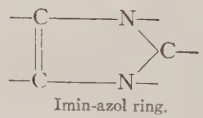
Calculation: $20 \times \frac{2}{\text{reading of unknown}} =$ mgs. of tyrosin in the amount of amino-acid solution taken for the analysis.

Group V. Heterocyclic Compounds. This group embraces the most complex and many of the most important α-amino-acids. The complexity consists principally in the incorporation of a heterocyclic ring, such as the imin-azol and the pyrrolidin rings into the molecule.

15. *l-Histidin, Beta-iminazole-α-amino-propionic Acid.*—The formula for histidin is given below. We have incorporated, also, a structural formula for the iminazol ring, which is very important for bio-chemistry, since it appears in some of the most characteristic substances of protid nature:



Histidin.



Imin-azol ring.

Histidin, then, is propionic acid ammoniated in the alpha position, with an iminazol ring atached to the beta-carbon atom. The relations of the iminazol ring to other configurations will be described during the course of the following pages. Histidin fed to a dog, causes increased excretion of urea in the urine. If we write the formula for urea thus:[1] , and incorporate a carbon chain, thus: , we may explain this phenomenon; the carbon chain may be derived from many sources. We shall encounter this ring again when considering the important protids which make up the nucleus of the cell.[2]

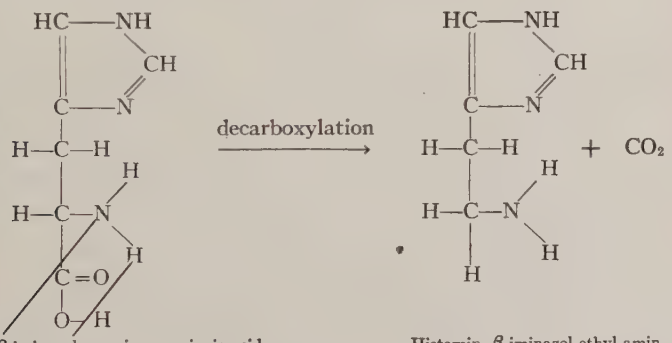
[1] Compare page 286.

[2] Page 329.

Histidin is probably the basis of the so-called Ehrlich[1] di-azo reaction, which is clinically used for the detection of urochromogen in the urine, a means of diagnosing certain diseases like tuberculosis.[2]

The percentage weight of histidin varies in different tissues. The average weight is 2.0 gs. per cent. in animal protids, but it may be much higher (11.0 per cent. in the globin in hemoglobin). On the other hand, it is absent in some protids, as in the protamin of the salmon spermatozoön. Hanke and Koessler found none in normal human liver.

Histamin.—We have learned[3] how tyrosin became converted into a substance with powerful pharmacological effects, tyramin. Another substance belonging to the same general group occurs in ergot; this is histamin, of which we have spoken. The method of conversion of histidin to histamin is the same as that of tryosin into tyramin. Decarboxylation:



Histidin, β-iminazol-α-amino-propionic acid. Histamin, β-iminazol-ethyl-amin.

Histamin has been called a "poison to the capillaries." It is the most potent of the amins. Given by mouth, it is toxic to herbivores, but not to carnivores. It is rendered non-toxic in the intestinal epithelium. Before stringent measures were enacted to prevent the distribution of the plant parasite, ergot, mentioned above, the host of which was rye and other cereals, many cases of gangrene appeared which were due to the histamin in the ergot accompanying the grains. When terminal gangrene occurs following the ingestion of ergot, it is

[1] Ehrlich, Paul, German chemist, who described many substances used in treating parasitic diseases like syphilis. The reagent of the "Ehrlich Di-azo reaction" is di-azo-benzene-sulphonic acid. See Chapter XV.

[2] The test for urochromogen is also made by causing it to become oxidized to urochrome by means of potassium permanganate, as in the Moriz-Weisz clinical test for the presence of tuberculosis. However, a positive test of either kind is not universally accepted as certain indication of the presence of the disease.

[3] Page 269.

due to the prolonged dilatation of the capillaries with associated constriction of the arterioles. The dilatation of the capillaries is due to the paralysis of the muscle whose function it is to diminish the diameter of the blood-vessels. The following table shows the characteristics and differences in the action of the three substances, epinephrin, tyramin, and histamin:

Substance.	Effect on uterus.	On blood-pressure.
Epinephrin	none	rise
Tyramin	none	rise
Histamin	contraction	fall

Confusion formerly existed in cases in which effects similar to those exhibited by histamin had been found, as following the administration of Witte's pepton.[1] It is now known that histamin is not necessarily present when such effects are found, for they may be caused by other agents, such as the secretion of the pituitary gland (pituitarin), pepton, etc. Amins are deaminized in the liver and in other tissues and hence become detoxicated. They are excreted in the same manner as the amino-acids tyrosin and histidin.

The Putrefaction of Histidin.—This process is due to bacteria of certain strains. It is comparable to similar changes of other amino-acids. The reactions are as shown at (1) on p. 275. Such substances are excreted in the urine to the extent of about 120–220 mgs. in twenty-four hours,[2] but some of the iminazole compounds are changed by disruption of the ring, shown at (2) on p. 275.

Acids Containing a Pyrrolidin Ring, Imino-acids:

16. *l-Prolin, α-pyrrolidin-carboxylic Acid.*—The formula for this amino-acid varies in many respects from that of others; its chemical relationships are evidently with the α-amino-acids. Sǿrensen synthesized it artificially from an aliphatic acid known as β-hydroxy-valerianic acid.[3] The latter has not yet been proved to be an hydrolysis product of the protids, yet this is quite possible. The formula is shown at (3) on p. 275.

Prolin is distinguished from all other α-amino-acids[4] by its ready solubility in strong ethanol. It is easily converted to a copper salt (blue) and, purified, white prolin may be obtained from the blue salt. Prolin is present in almost all protids. The casein of cow's and

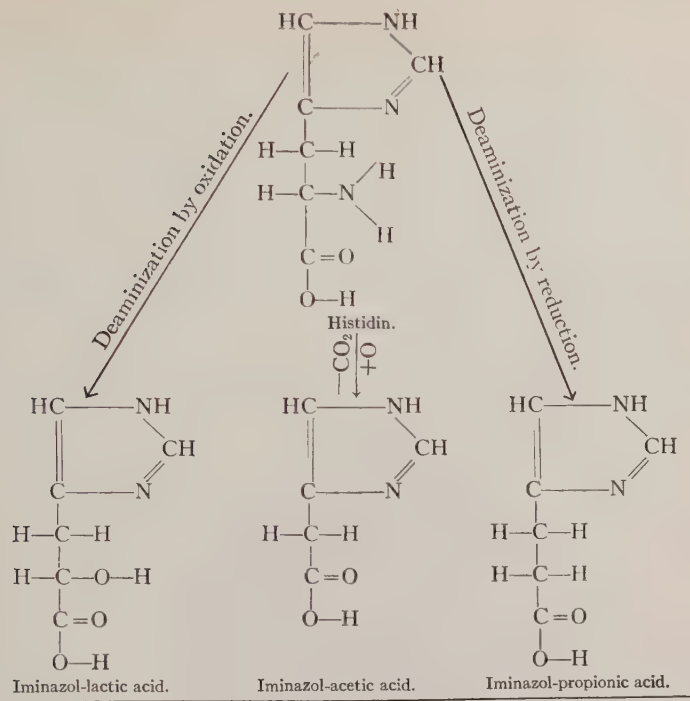
[1] Page 237.

[2] Page 747.

[3] Recently evidence that prolin and the following *l*-hydroxy-prolin are synthesized during analysis of such substances as gelatin has been given. See Knaggs, J. and Schryver, S. B., Biochem. J., vol. 18, page 1102, 1924.

[4] Except oxyprolin, a relation of prolin; see page 276.

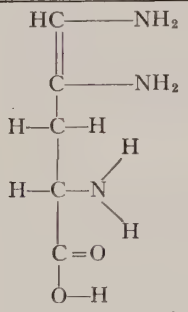
(1)



Histidin.

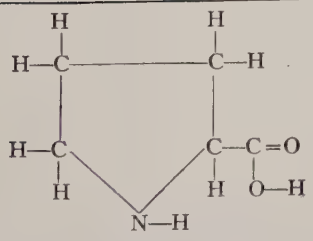
Iminazol-lactic acid. Iminazol-acetic acid. Iminazol-propionic acid.

(2)

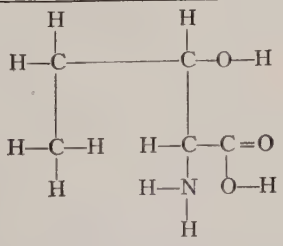


Diamino-azo-propionic acid.

(3)



Prolin, α-pyrrolidin carboxylic acid.



β-hydroxy-valerianic acid.[1]

[1] The formula is written to show how it is convertible into prolin by the loss of a molecule of water.

goat's milk has prolin in the molecule, but it has not been found in the casein of human milk. It is present in gelatin and closely allied substances in fairly large amounts. By conversion into lactic or to keto acids, prolin contributes to the formation of glucose. It resembles glutamic acid in some respects which have been discussed under lactam formation.[1] Since prolin contains the pyrrolidin ring it may contribute to the formation of hemoglobin.

The vapors of boiling prolin solutions give bright red color when brought into contact with a match-stick wetted with hydrochloric acid.

17 *l-Oxyprolin*,[2] *Gamma-hydroxy-α-pyrrolidin-carboxylic Acid.*— If oxyprolin be treated with a reducing agent, like hydriodic acid, it

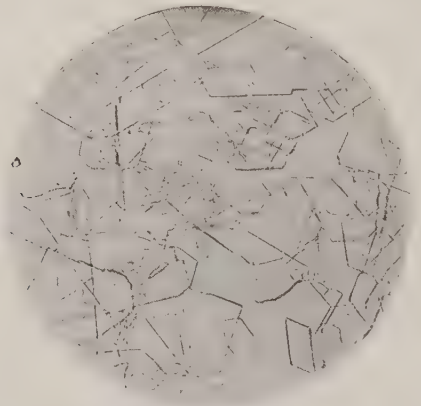
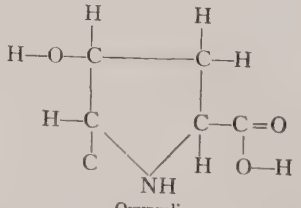


Fig. 107.—Tryptophan. Thin rhombic plates and hexagonal crystals. Magnification as in Fig. 96. (After Keenan in Journal of Biological Chemistry.)

is converted into prolin. Theoretically, the hydroxyl, distinctive of oxyprolin, may occur on either the beta or gamma carbon atom, but is customary to consider the compound found in the hydrolysis products of protids as a gamma compound:



Oxyprolin
γ-hydroxy-α-pyrrolidin-carboxylic acid.

[1] Page 257.

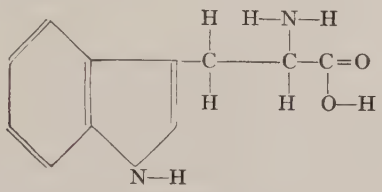
[2] It is better to call this compound "hydroxyprolin."

Oxyprolin is separated with difficulty from accompanying amino-acids. For this purpose all othe amino-acids must be removed by crystallization or other means. Oxyprolin, like prolin, contains the pyrrolidin ring which is also characteristic of hemoglobin. Although little is known regarding the manner of synthesis of hemoglobin in the hemopoietic[1] part of bone (the marrow), it is probable that this ring is not synthesized in the marrow, but is brought there. The food, therefore probably furnishes this part of the coloring-matter of the blood. Of the pigments in the plant series from which our food is derived, chlorophyl, or green coloring-matter, is most similar to hemoglobin. Two sources, then, are available for the pyrrolidin ring in our food: (1) The amino-acids and (2) plant pigments.

Like prolin, oxyprolin differs from other amino-acids in being soluble in strong alcohol.

Acids in Which the Indol Ring is a Part of the Molecule:

18. *l-Tryptophan, Beta-indol-α-amino-propionic Acid.*—The formula of this important acid is:

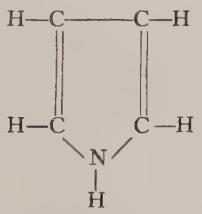


Tryptophan
β-indol-α-amino-propionic acid.

The molecule consists of:

(1) A homocyclic ring (benzene) } Indol ring.
(2) A heterocyclic ring (pyrrol) }
(3) An aliphatic amino-acid, propionic acid.

The pyrrol ring attached to the benzene ring is an unsaturated pyrrolidin ring:



Pyrrol ring.

Tryptophan is responsible for many of the reactions used in the detection of protid.[2] It is also responsible for certain putrefactive

[1] Greek *'aima* (haima), blood, and *poieo*, to make; the blood-forming organs.

[2] Page 312.

products of bacterial action. Since the presence of such products in urine indicates the degree of putrefaction in the alimentary tract to a certain extent, the indican test, devised to detect their presence in urine, is important. The process of putrefaction here is similar to what we have encountered before, namely, decarboxylation, etc.

Tryptophan is never present in conspicuous amounts in protids of any kind, but nevertheless it is of signal importance in metabolism. It is not present in the corn protid, zeïn, and for that reason this protid has been utilized to study the effects of feeding protids which lack certain amino-acids. Pure gelatin lacks tryptophan. An animal fed upon gelatin as the source of protid cannot be brought into nitrogenous equilibrium, as has already been stated,[1] but if tryptophan is added in minute quantities to the diet, this equilibrium is established at least for short periods.[2] Similar statements may be made concerning zeïn.

We know that acid hydrolysis products of protid, when purified, cannot be made to take the place of the original protid, whereas enzyme-digested protid products can. This is explained by the fact that in acid hydrolysis tryptophan is changed (deaminized, oxidized, or racemized), and hence is no longer available.

Folin and Looney's Quantitative Method for Tryptophan.—Principle: The residue left from the separation of tyrosin[3] is treated similarly to the manner in which tyrosin is determined. The difference in method concerns the treatment of the standard.

Procedure: Dilute the residue in the centrifuge tube which is left after the supernatant liquid carrying the tyrosin has been removed with 5 per cent. sulphuric acid solution to make 10 mls. volume. Stopper, invert, shake, and centrifuge. Decant it, retaining the residue. Add to the precipitate and to the precipitate obtained by similar treatment of a standard tryptophan solution containing 1 mg. tryptophan, 10 mls. of distilled water, then stopper both tubes and shake them vigorously. Immediately add 4 mls. of 5 per cent. NaCN solution, stopper the tubes again, and mix. Transfer the contents of the tubes separately to one of two 100-ml. volumetric flasks to make

[1] Page 253, note 1.

[2] The reason for this modifying clause to the statement that restoration of nitrogenous equilibrium may be made is that the experiments upon which the statement is based involved the restoration only of tryptophan, and two other factors were left out of account, namely: (1) Other amino-acids absent from gelatin (cystin, isoleucin, and tyrosin) and (2) vitamins, page 138. Tyrosin was added in certain experiments, but in these no attention was directed to other amino-acids nor to vitamins.

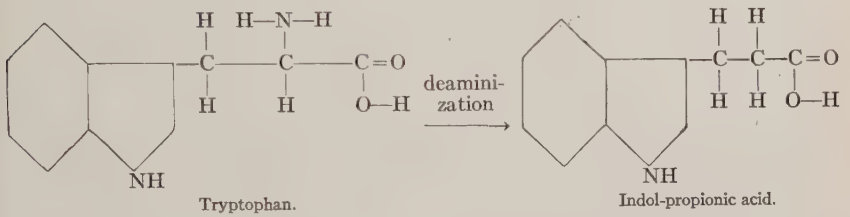
[3] Page 271.

a volume of about 50 mls. in each flask. Add to each flask 20 mls. of saturated Na_2CO_3 solution and, while agitating the contents, add 2 mls. of the phenol reagent.[1] Let stand half an hour, make up to the mark, and compare in a colorimeter, using the standard at 20° C.

Calculation: $\frac{20}{\text{Reading of the unknown}}$ = mgs. of tryptophan in the sample of amino-acid mixture taken.

Tryptophan undergoes change in two ways:

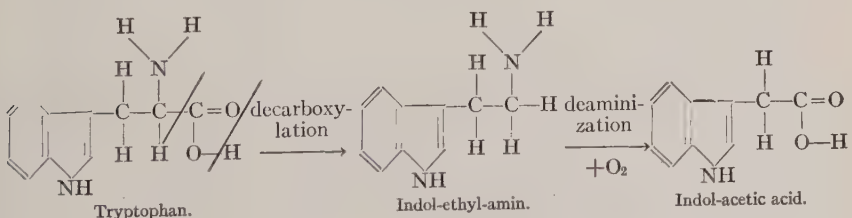
(1) In the body it may become deaminized, forming indol-propionic acid as shown in the following equations:



Tryptophan. Indol-propionic acid.

Then the indol-propionic acid undergoes β-oxidation.[2] Acetic acid is split off and the residual indol compound is then conjugated with some substance like glucuronic acid[3] and excreted in this detoxicated form. The acetic acid is used for further oxidation, being burned ultimately to CO_2 and H_2O. While the liver is concerned in such disruptions of molecules, all tissues share these properties to some extent.

(2) In the intestine it is subject to bacterial action. Here the process leading to simpler compounds is chiefly that of decarboxylation:

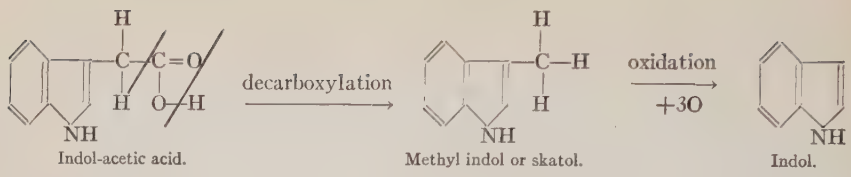


Tryptophan. Indol-ethyl-amin. Indol-acetic acid.

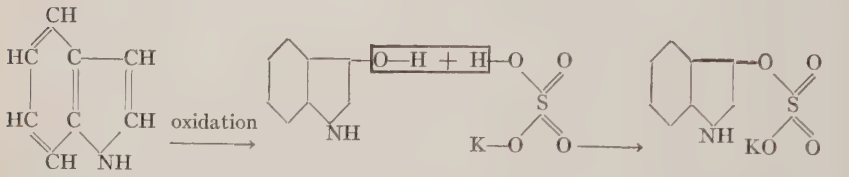
[1] Appendix.

[2] Page 265.

[3] The acid is derived from glucose and belongs in the same series as galacturonic acid (pages 157, 281 and 282).

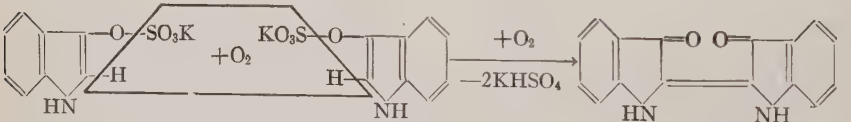


Indol-acetic acid. Methyl indol or skatol. Indol.



Indol (showing complete atomic equipment). Indoxyl. Potassium acid sulphate conjugating with indoxyl. Indoxyl-potassium sulphate (indican).

Indican, or the potassium salt of indoxyl-sulphuric acid, is excreted in the urine as one of the "ethereal sulphates." The indican test involves an oxidation of the indican to make the colored substance indigo, which is formed by the removal of two molecules of potassium acid sulphate one from each of two conjugated molecules of indican, as follows (indican reaction):



Conjugation of two molecules of indican to form one of indigo. Indigo.

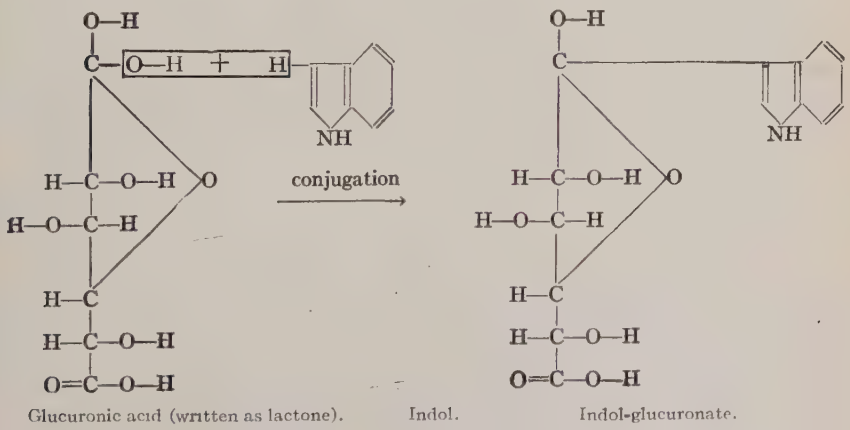
In the test which is given in the section on Urinalysis[1] the oxidizing agent is some substance like bleaching-powder, CaCl(O.Cl) ferric chlorid in HCl solution,[2] cupric sulphate in HCl solution, potassium persulphate $(KSO_4)_2$, or perchlorate, $KClO_4$. The usefulness of the test as an index of the degree of putrefaction in the intestine and therefore to the activity of that organ has already been commented on. If feces accumulate for any length of time in the intestine, tryptophan undergoes changes due to the action of bacteria.[3] Indol results and is absorbed into the blood through the intestinal wall, is conjugated with sulphuric acid or its salt, and is then excreted as indican through the kidneys. If there is only a slight retention of the feces in the intestine, skatol is formed, which imparts to the feces an

[1] Page 758.

[2] This is used in the method of Obermayer (Fr. Obermayer, a Vienna physician and biochemist), page 758.

[3] Page 279.

extremely disagreeable and characteristic color. Skatol may even appear in the breath during fecal retention. Retention of the feces in the lower portion of the large intestine does not contribute to the indican of the urine to a marked degree, but obstruction in the region of the ileocecal valve and especially in the small intestine above it, causes indicanuria. In cases of gangrene or of purulent masses like cancer indican appears in the urine. These facts lead to the assumption that tissue enzymes rather than bacteria cause the various chemical processes just described which produce indican from tryptophan. Although indol is not highly toxic, it has been held responsible for many ills affecting mankind; and there is convincing evidence that its continued administration will result in certain lesions (renal changes, affections of the adrenal glands, etc.). Lee[1] has found that indol produces typical fatigue effects in muscles. Experimentally, it induces accelerated cell division, leading to rapid proliferation of epithelial tissue. Skatol[2] also has the property of inducing or accelerating cell growth in experimental conditions. Skatol, like indol, becomes conjugated with sulphuric acid salts to form skatoxyl-sulphuric acid after previous oxidation to skatoxyl, which is analogous with indoxyl. Skatol is formed in smaller quantities than indol; it is not in the chain of chemical changes giving rise to indol. If the sulphuric acid salt in the liver and other organs where conjugation takes place is insufficient, both indol and skatol conjugate with one of the acids of glucose oxidation, glucuronic acid,[3] as follows:

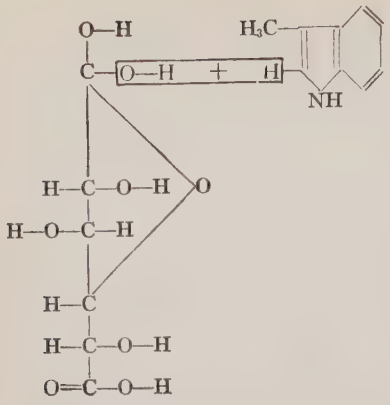


Glucuronic acid (written as lactone). Indol. Indol-glucuronate.

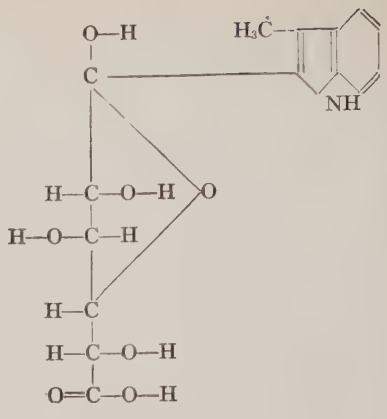
[1] Lee, F. S., see page 9.

[2] Other substances, like cholesterol, have been found to be associated with increased proliferation of certain cells.

[3] Page 279, note 3.



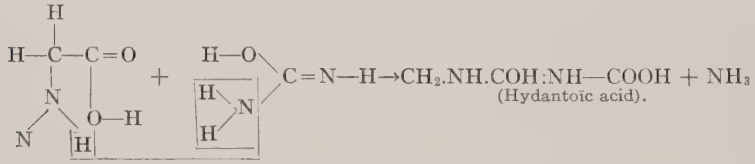
Glucuronic acid. Skatol.



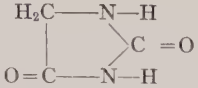
Skatol-glucuronate.

These conjugations are very important, since (1) they represent the way in which the unopened aromatic ring leaves the body, and (2) they show how exceedingly necessary protective processes of dextoxication by conjugation occur in typical cases.

Combinations of Amino Acids.—Uramino-acids are combinations of urea and amino-acids:



Such substances are called *ureids* (Greek urea + *'oidos*, like). They are homologous with amino-acids for they are linkages of an ammonia compound (urea) with acids. If such an uramino-acid as the one given above be treated with a mineral acid, a *lactam* (page 257) is formed as an hydroxy-acid ureid:



Cylic ureid of glycolic acid (a hydantoïn).

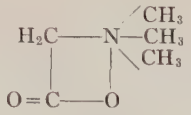
Compare allantoin (page 338).

Straight-chain acids, like the hydantoïc acid just described are known as *hydantoïc acids*, while the cyclic forms are called *hydantoïns*.[1] Johnson[2] has recently demonstrated an hydantoïn nucleus in protids.

[1] From Gr. *hydor*, water + *allantoïn* (page 338, note 3), since hydantoïns are derived by reduction from allantoïn.

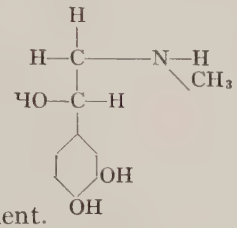
[2] Johnson, T. B. (Professor of Organic Chemistry, Yale University). Paper read before the National Academy of Science, Philadelphia Meeting, Dec. 1926.

Betaïns[1] are alkyl (page 222) derivatives of amino-acids. Thus, the tri-methyl derivative of glycocoll is:



Betaïn.

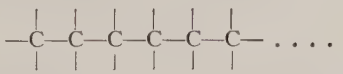
Somewhat akin to such compounds is epinephrin (page 267); written thus:



The resemblance is evident.

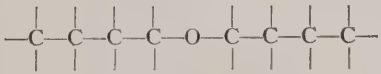
THE MANNER OF LINKING AMINO-ACIDS INTO PROTIDS

Amino-acids are ammoniated fatty acids, and therefore consist of a chain of carbon atoms. When these acids are formed into protid, how are they combined? Is one acid linked with another through carbon atoms to make a long chain of carbon atoms as follows?



The difficulty with such a scheme is that it would make protid a very long branched chain with side-chains of carbons of about the same value as the carbon atoms in the main chain. There would be, therefore, no reason chemically why the chain should break up into separate amino-acids when protid is acted upon by acid or enzymes.

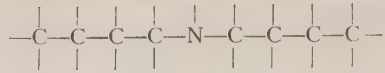
Another possibility is that the linkage is through an oxygen atom as follows:



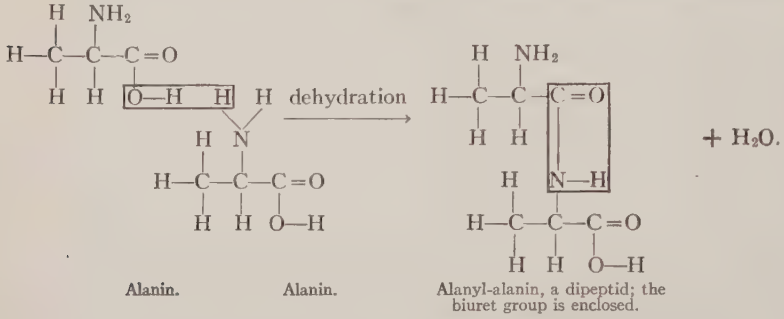
This ether-like linkage would require free hydroxyls such as occur in glucids and in glycerol in the case of the fats. However, serin, oxyprolin, and tyrosin are the only amino-acids which bear such radicles and, therefore, this theory would not explain the linkage of many other forms of amino-acids into protid. The bonding could not be by way of carboxyl radicles to carboxyl radicles, for such combinations are impossible.

[1] So called from the original source, the sugar beet, called by botanists *Beta vulgaris*.

A third possibility is linkage by way of a nitrogen atom:



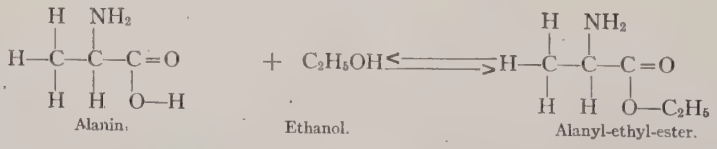
We may take as an example the linkage of a simple amino-acid like alanin with another molecule of the same kind of amino-acid:

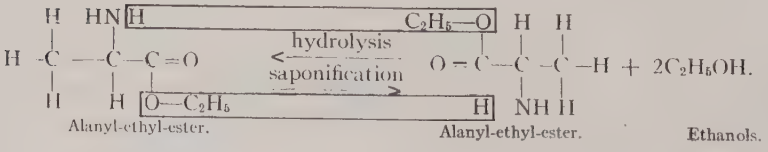


We have indicated by solid lines the nitrogen atom and its adjacent carbon atoms linking the two amino-acids, alanin and alanin, into the di-peptid, alanyl-alanin. The process is one of hydrosynthesis, a molecule of water being eliminated for each amino-acid added. Fischer succeeded in synthesizing a polypeptid consisting of 18 and Abderhalden one of 19 amino-acids.

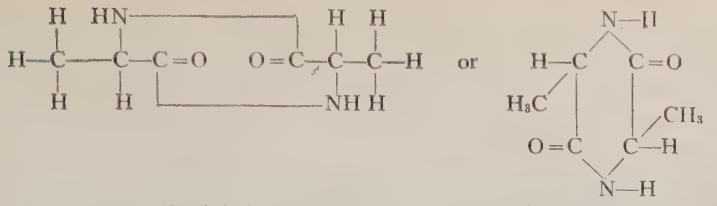
Abderhalden[1] believes that a simple linkage like that just described is unlikely since dissociation of the protid molecule requires such vigorous chemical means.

It is probable that the amino-acids are linked in a cyclic form. We may call this method the *anhydrid linkage*. Such cyclic anhydrids have been known for many years, but it was assumed that ordinary amino-acids became transformed into these compounds on standing, especially at higher temperatures. An example of such a transformation is given, the union between two adjacent amino-acids being effected by converting them into esters:



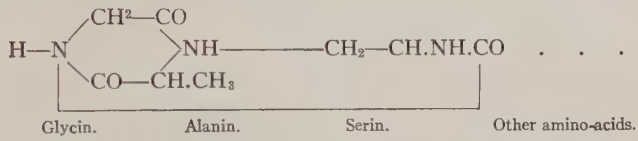


[1] Page 240.



Alanyl-alanin. Di-methyl-di-keto-piperazin.[1]

All of the reactions are reversible.[2] The reader will observe that in these substances the linkage is the same as in the preceding scheme, namely, by way of the biuret bond, HNCO. The following example shows how other amino-acids may become a part of the protid, through the diketopiperazin linkage:



Glycin. Alanin. Serin. Other amino-acids.

Proof of the Nitrogen Linkage in Protids.—We shall present two reasons for believing in this form of linkage. These reasons are capable of practical demonstration by the student, and each method involves a fundamental laboratory procedure.

(1) *The Biuret Reaction:*

EXERCISE 33.—Place 5 mls. of egg-white (1 per cent.) in a test-tube and add half its volume of 10 per cent. sodium hydroxid solution. Mix. Now add 1 drop of 0.5 per cent. cupric sulphate solution while agitating the contents of the tube and note the appearance of a violet color in the protid—NaOH—$CuSO_4$ solution. This is known as the biuret color and the test is the most characteristic one for the presence of protid.

Gies'[3] *Modification of the Biuret Test.*—The reagent is made up in stock solution.[4] The test is performed simply by adding carefully to form a layer by means of a pipette one volume of the reagent to one of the unknown solution. At the zone of contact a violet color appears if there is protid in solution. The densities of the unknown

[1] The "keto" radicles are the —C=O groups in positions 2 and 5.

[2] Enzymatic syntheses of protid-like substances have been reported recently by Wasteneys (Borsook and Wasteneys, Jour. Biol. Chem., vol. 62, pages 15 and 633, 1923 and 1925) and by Abdenhalden (Abderhalden E. and Sickel, H. Zeitschr. f. physiol. Chem., vol. 158, page 139, 1926).

[3] Gies, William J., Professor of Biochemistry, College of Physicians, Columbia University, New York (see Fig. 133; also page 24).

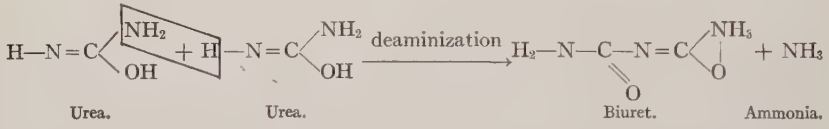
[4] Appendix.

and of Gies' reagent are similar, and zoning occurs only when care is taken.

The principle of the biuret test may be illustrated, practically, as follows:

EXERCISE 34.—Repeat the foregoing Exercise, using, in place of the protid solution, a few dry crystals of urea. Drop them into a dry test-tube. Heat the tube gently in the flame until whitish vapors appear in the tube; then dilute the residue with 5 mls. of the alkali as before and add cupric sulphate.[1] A biuret color develops in the solution.

The name "biuret" is given to the substance which is formed on heating urea in the test-tube; two molecules of urea condense to form the substance biuret (bi-urea) as follows:



In the formula given above for biuret there are two configurations similar to that linking two alanyl radicles in the di-peptid, alanyl-alanin, described on page 285. Alanyl-alanin does not give the biuret reaction, while biuret does. It is the presence of *two* groups equivalent to H—N = C = O that is responsible for the reaction of biuret. Other configurations of a similar sort give the reaction:

$$CS-NH \qquad C.NH-NH \qquad CH_2-NH$$

Therefore, the configuration which we have used before[2] when present twice in a molecule is responsible for the test, and although in the di-peptids but one such radicle appears (as in alanyl-alanin) and the biuret reaction is negative, one may make it positive by adding a third amino-acid to make a tri-peptid.

Logically, we must assume that the linkage in the di-peptid is the same as in the tri-peptid, since the same chemical procedure is passed through in making both kinds of acids.

(2) *The Comparison of Amino-nitrogen and Total Nitrogen in Protid.*— If we are correct in believing that the natural linkage of amino-acids into protids involves the configuration HNCO, then we should expect only a small amount of nitrogen such as is present in isolated amino-acids to be given off from the protid. This would be

[1] Or use Gies' biuret reagent as described.

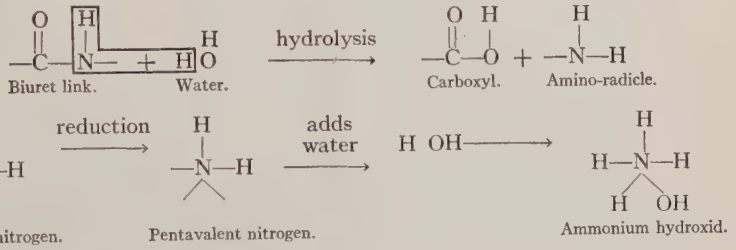
[2] Page 284.

in the form of amino-nitrogen, NH_2. However, it is probable that the protid nitrogen is largely in the form of imino-nitrogen, NH. We shall proceed to demonstrate this fact by making two quantitative determinations as follows:

(*a*) Determination of the total nitrogen by the Kjeldahl method.

(*b*) Determination of the amino-nitrogen, NH_2, by the nitrous acid method of Van Slyke.

(*a*) *The Kjeldahl Method*[1] *for Total Nitrogen.*—The reactions as far as protid is concerned are as follows:



$$2\,NH_4OH + H_2SO_4 \longrightarrow (NH_4)_2SO_4 + 2H_2O$$

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2NH_4OH + Na_2SO_4$$

EXERCISE 35.—Obtain from the stock-room a weighed sample of pure casein containing about 1 g. of the substance, the exact weight of which is known. Transfer it quantitatively[2] to the mouth of a 700-ml. Kjeldahl flask[3] and wash down with about 5 mls. of a 1 per cent. sodium or lithium carbonate solution, which is capable of dissolving the casein. Now add to the flask about 25 mls. of concentrated sulphuric acid and a crystal of cupric sulphate. Place the flask on the digestion shelf (Fig. 109), the neck of the flask being inserted into one of the round apertures in the lead pipe designed to carry away the fumes which escape from the flask. Light the burner beneath the flask and with an average flame heat the flask until the contents begin to boil; then add 5 gs. crystalline potassium sulphate, and continue heating as actively as possible until the contents have passed through a brownish-black stage, a yellow stage, and finally a clear blue. Remove the flask with an asbestos glove and wash the contents around the interior of the flask until all carbonaceous material and extra sulphate adhering to the neck and sides of the flask are

[1] For other reactions involving the Kjeldahl method see Chapters XV and XVI.

[2] By this term is meant that absolutely all of a sample is transferred without loss.

[3] Page 289 and Fig. 108. The long neck of the flask is to prevent too great concentration of the contents of the flask by evaporation.

added to the liquid contents at the bottom of the flask. Replace the flask on the digestion shelf and let the contents boil for one hour or longer. Then allow the flask to cool until the hands will tolerate the temperature of the glass. Cool further under a stream of cold water from the tap, allowing small amounts of tap-water to enter the mouth of the flask until the contents are diluted to about 400 mls. Now add some pebbles to prevent bumping during the latter part of the next stage of distillation.[1]

Fig. 108.—The Kjeldahl type flask. The long neck is to prevent too great concentration of the contents during boiling and to serve as a condenser.

Fig. 109.—Folin's apparatus for digestion of substances in the determination of total nitrogen by the Kjeldahl method.

Add about 5 mls. of an indicator,[2] which should show that acid is present in the flask. Set the flask aside until the contents settle and give your attention to the preparation of the standard solution which is to receive the ammonia distilled from the flask. Take a clean pint milk-bottle and place in it exactly 125 mls. of decinormal acid[3] by means of a pipette or burette. Adjust the bottle so that the glass tube from the lower portion of one of the condensers of the

[1] The following treatment may be delayed at this point, but if the next step is taken, the process cannot be interrupted until the end of the distillation, which requires about one and one-half hours. Bits of broken glass, small lumps of pumice, or glass beads may be used in place of pebbles.

[2] Methyl red is desirable, but sodium-alizarin sulphonate, congo red, or methyl orange may be used.

[3] It is immaterial what kind of acid is used, save that it must be a strong acid like H_2SO_4, HCl, etc. (See page 43.)

condensing apparatus dips below the surface of the acid in the bottle (Fig. 111, M), add about 2 mls of congo red, or whatever indicator was used above, to the acid. Now carefully and without agitating the contents incline the Kjeldahl flask slightly and pour down the inside of the neck about 125 mls. of concentrated NaOH solution (50 per cent.) so that the alkali goes to the bottom of the flask. The acid should float upon this whitish alkali layer and may be distin-

Fig. 110.—Principle of Kjeldahl distillation. The Kjeldahl flask (right) contains the nitrogen in the form of ammonia, which is distilled into the Erlenmeyer flask (left) which contains known quantity of acid of known normality.

guished by the characteristic color reaction of the indicator. You may now place the flask upon the condenser. Fit the neck of the flask with the rubber stopper containing the glass tube of the Reitmeyer bulb[1] attached to the blocked tin tube of the condenser. After the stopper is firmly in place, slowly rotate the contents of the flask until the reaction is alkaline as shown by the indicator present. This is due to the neutralization of the acid by the alkali.[2] Immediately after this the Bunsen burner beneath the flask must be lighted and the flame adjusted so that the contents will come to a boil in about five

[1] Figure 111. Reitmeyer, a German chemist. The purpose of the bulb is to catch any of the contents of the flask which happen to boil up through the neck of the flask; the caustic alkali from such source would partly or completely neutralize the acid in the milk bottle and destroy the accuracy of the determination.

[2] Care must be taken that this is done, for if the flame is applied to the flask before the acid and alkali have mixed, the contents will be forced through the neck of the flask or the flask will be shattered, which is an element of danger to the manipulator, besides spoiling the determination.

minutes. At this step strict attention must be given to the apparatus so that the contents do not boil up suddenly and overflow. The contents should come to a boil gently and the boiling continued until the milk bottle is three-quarters full.[1] To stop the process of distillation, *first remove the milk bottle*, permitting the glass tube dipping into it to rest in an inclined position upon the mouth of the bottle; then, and then only, extinguish the flame of the burner beneath the flask.

Now, using the total contents of the milk bottle, titrate with standard alkali and determine how much of the acid has been neutral-

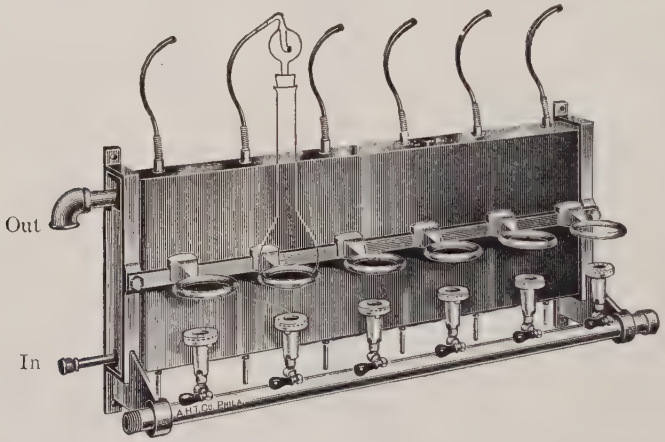


Fig. 111.—Folin type distilling shelf for total nitrogen determinations by means of the Kjeldahl method. A Kjeldahl flask is shown in place. Cold water must pass through the condenser reservoir from the inlet, In, to the outlet, Out.

ized by the ammonia distilled from the flask. Calculation: Multiply the *volume* of nitrogen solution (0.1 n. acid $\simeq$ 0.1 n. N) by the *mass* of N in 1 ml. 0.1 n. N solution; this is 0.0014 g. The result is grams of nitrogen in the amount of substance taken (page 292).

The average amount of nitrogen in protid is about 16 gs. for 100 gs. of protid, or, expressed in decimal fractions,

$$\frac{100}{16} = 6.25$$

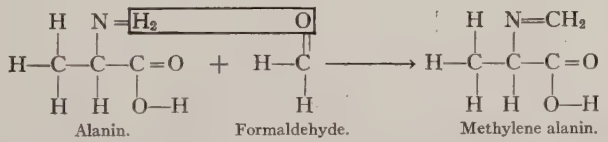
If the amount of nitrogen in a given sample of protid is known and is multiplied by the average percentage of nitrogen determined in protid, that is, 6.25, an approximation of the number of grams of protid corresponding to that number of grams of nitrogen will be obtained.

[1] If at any time the contents of the milk-bottle begin to turn alkaline, another 25-ml. portion of decinormal acid must be added as quickly as possible, for the appearance of an alkaline color indicates that there is not enough acid in the bottle to neutralize the ammonia distilling over.

The factor 6.25 is a convenient figure whereby one may tell approximately how much protid corresponds to a given amount of nitrogen.[1] Having determined the total amount of nitrogen in the sample of casein, let us proceed to estimate the amount of amino-nitrogen in a sample of the same preparation of casein.

(*b*) *The Determination of the Amino-nitrogen by the Nitrous Acid Method.*—There are several excellent methods for estimating, quantitatively the amount of $-NH_2$ nitrogen in solutions. For the purpose of proving the small amount of this form of nitrogen existing freely in protids, we shall use the following method first as preliminary to the Van Slyke procedure following.

Preliminary Determination by the Formol-titration Method of Sørensen.[2]—Principle: Amino-acids unite with either acids or bases. They are amphoteric[3] to a certain degree, that is, they do not show pronounced alkalinity or acidity, for the amino-portion (basic) is balanced against the carboxylic (acid). Therefore, if either portion is neutralized, or altered so that it no longer counteracts the effect of the other portion, it is possible to titrate the acidity or basicity of the latter. In the method of Sørensen this is accomplished by methylating the amino-radicle by means of formaldehyde. The reaction is as follows, using alanin as an example:



The methylene compound, by way of its carboxyl, is capable of neutralizing a base, like decinormal NaOH. There is in the majority of amino-acids[4] but one carboxyl radicle; hence the number of amino-

[1] This factor is constantly used in studies on metabolism. The nitrogen appears in the urine chiefly as urea, uric acid, ammonia, creatinin, and creatin. These result from the breaking down of the tissues or from foods. The nitrogen determined multiplied by the factor 6.25 gives approximately the number of grams of protids, and this serves as an index of body tissue catabolism.

[2] Page 39.

[3] Page 108.

[4] The exceptions are the dicarboxylic acids, which may be called the acid compounds, while the di-amino-acids and histidin compose the basic forms; the remaining amino-acids (mon-amino-carboxylic acids), including those with one amino and one carboxylic radicle, and also tyrosin and tryptophan, may be termed the neutral acids (amphoteric). Cystin, although it has two carboxyls, has also two amino radicles to balance them. No amino-acid is strictly amphoteric, as there is a predominance of acidity or basicity in all when delicate *p*H measurements are made.

acids which may have free carboxyls is determined by finding how many equivalents of OH must be added to neutralize the hydrogen of the carboxyls. The calculation is the same as in the Kjeldahl method for total nitrogen.

Exercise 36.—*Preparation of Neutral Formalin.*—Commercial formalin contains formic and other acids as impurities; therefore, in this method, it is necessary to neutralize a supply of formalin. To 50 mls. of commercial formalin solution add 3 drops of phenolphthalein solution as indicator and titrate with decinormal sodium hydroxid solution until a faint pink color appears. Then add 1 drop of the alkali. Before titrating, place 10 mls. of this solution in a beaker or test-tube and have it near the vessel which contains the unknown, to serve as a color control to which titration should be carried. *Procedure:* Obtain a sample of casein, weigh carefully, and record the exact weight. Then suspend in 20 mls. of distilled water in a beaker. Add 3 drops of phenolphthalein solution as indicator and titrate with decinormal NaOH solution to the color of the sample prepared above. You have neutralized any acid which may be present.[1] Next add 10 mls. of the neutralized formalin by means of a volumetric cylinder; the pink color is discharged. Titrate with 0.1 normal NaOH, drop by drop, until the solution is neutralized, as indicated by the reappearance of the pink color of the phenolphthalein.[2] Note the number of mls. of 0.1 normal alkali used. Calculation: Here, as in the Kjeldahl method, 0.1 normal NaOH is equivalent to 0.1 normal H^+ and each H^+ corresponds to one carboxyl; there is one carboxyl to each atom of nitrogen in the monamino radicle NH_2.[3] A decinormal nitrogen solution contains, per ml. of solution, 0.0014 g.[4] If w mls. of decinormal alkali are used in the titration, there will be $w \times 0.0014$ gs. of nitrogen in the sample of casein taken.

The average amount of free amino-nitrogen in protid is 6.8 gs.[5] per 100 gs. of protid. For our purpose this demonstrates that the

[1] The instructor should insure that the sample is neutral.

[2] For the end-point in formal-titration see Northrup, J. H., Jour. Gen. Physiol., vol. 9, page 767, 1926.

[3] This must be modified when other forms of amino-acids are being analyzed; the method does not distinguish acids qualitatively.

[4] Page 290.

[5] Not to be confused with the percentage of nitrogen in protid, which is 16 per cent. or, stated in decimal fraction, 6.25. The corresponding factor for free amino-nitrogen is $\frac{100}{6.8} = 14.8$.

amino-acids are so linked up with each other that there are few amino-groups and carboxyls. It does not show the exact number of such radicles in protid, because there is reason to believe that protid is linked with other substances such as glucose, lecithin, etc. Such linkage probably occurs between the carboxyl-radicles, although there are other possibilities, and it is not positively known how extensive such association is. However, it is evident from the following exercise that whatever the form of linkage of the separate amino-acids in the protid molecule, it may be possible by hydrolysis to separate them completely from each other. Comparison will be made in the following Exercise between the data derived in Exercise 34 and those obtained when the amino-nitrogen is derived from separate amino-acids after hydrolysis:

Exercise 37.—*Preparation of the Hydrolysis Products.*—To 10 gs. of dry, purified casein[1] in a 100-ml. Florence flask fitted with a

Fig. 112.—The Hopkins reflux condenser. Cold water from the tap enters the left-hand tube at the top and leaves by the upper right-hand tube. The larger side-tube is to relieve the pressure within the condenser.

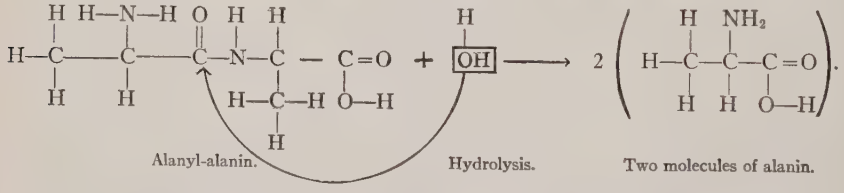
condenser (Fig. 112) add 25 mls. distilled water, mix well, and then add 3 mls. of concentrated sulphuric acid. Place on a wire gauze over a Bunsen burner, and boil gently for a period of three hours, interrupting the hydrolysis when necessary, but resuming it at the beginning of the following period. It is necessary to add a small amount of water occasionally to keep the solution at its original volume, so that it will not become too concentrated. At the end of the boiling make the digest up to 100 mls. by means of distilled water

[1] The casein should be of the same sample as that used in the previous Exercise.

and filter off the black or brown residue from the liquid part of the digest, saving both portions.

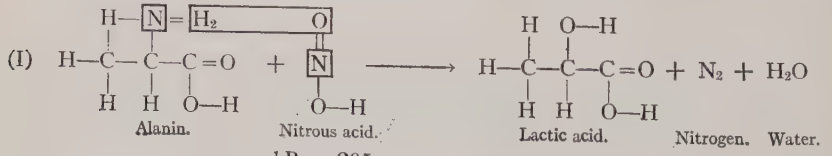
Filtrate: Neutralize the H_2SO_4 by means of barium carbonate solution (litmus). Filter. Use this filtrate for the determination of amino-nitrogen by the Van Slyke procedure given below.[1]

Residue: For the purpose of comparison, which cannot be made quantitatively exact in class work, this fraction should be subjected to further hydrolysis, which may be accomplished by noting the volume of the material and adding 5 mls. of concentrated H_2SO_4, then placing the digest in an autoclave for two hours, after which the above neutralization is repeated. Van Slyke or Sørensen determination may now be made as before. After the determination has been made by either method, it is evident that amino-nitrogen has greatly increased during hydrolysis, which indicates that the molecule of water, which was abstracted from the amino-acids during their synthesis into protid, has been restored to them, so that the imid, NH, nitrogen becomes converted into amino-nitrogen, NH_2 and the carboxyl is completed by the addition of the rest of the molecule of water, the hydroxyl. This is the process of digestion (alanin):



Each separated molecule of mon-amino-acid has a free carboxyl. When the formaldehyde methylates the amino-group, destroying its alkalinity, the titration gives the number of amino-acids, by giving the number of carboxyls.

Exercise 38.—*The Van Slyke Method for Gasometric Determination of Aliphatic Amino-acids.*—This exercise may be done by the student himself, if apparatus is available, or it may be demonstrated by instructors to small groups of students. Principle: Nitrous acid[2] deaminizes the amino-acid and adds its own nitrogen to make a molecule of nitrogen, $N \equiv N$, or N_2 (alanin):



[1] Page 295.

[2] HNO_2 or H—O—N = O.

The nitrous acid is readily and conveniently obtained by treating the solid sticks of potassium or sodium nitrite, $NaNO_2$, with acetic acid, $CH_3.COOH$:

(II) $Na—O—N=O + CH_3.COOH \longrightarrow H—O—N=O + CH_3.COO.Na.$
Sodium nitrite. Acetic acid. Nitrous acid. Sodium acetate,

Besides these products, carbon dioxid and other gases, especially nitric oxid, NO, are produced, and inasmuch as the procedure is one of gas analysis, gases besides nitrogen must be absorbed. This is accomplished by means of a solution of potassium permanganate, a strongly oxidizing reagent, and alkali, like NaOH. Carbon dioxid is removed by the alkali, to form sodium carbonate, while the colorless NO undergoes reaction with the permanganate:

$NO + KO_2.MnO_2 \longrightarrow KNO_3 + MnO_2.$
Nitric oxid. Potassium permanganate. Potassium nitrate. Manganese dioxid.

Some NO takes on, during the early stages of the process, atmospheric oxygen and becomes the yellow-brownish peroxid, NO_2; this is converted to nitrous acid in the Hempel pipette.[1]

Determination.—The apparatus:[2] Note that there are three principal parts to the apparatus: (1) The deaminizing bulb (Fig. 115, B), with its attendant burettes for filling it (A for the unknown, C for the reagents, and L for caprylic alcohol, phenyl ether, etc., which are capable of lowering surface tension and preventing foam). (2) A nitrometer (Fig. 116), resembling an inverted burette. (3) Absorption apparatus, a Hempel[3] pipette (Fig. 113, J and Fig. 114) containing two intercommunicating bulbs which are filled with an alkaline permanganate solution.[4]

Procedure: (1) Generation of the nitrous acid: Close cocks *x* and *y*; open *d* to the drain. Into burette *c* introduce about 10 mls.[5] of sodium nitrite solution and then open *e* so that the nitrite solution flows into the deaminizing bulb *b*. Close *e* again and place about 3 mls. of glacial acetic acid in *c*. Now open *e*, whereupon the acid and nitrite solution mix, with accompanying evolution of gas.[6] Close *d*,

[1] Figure 113, J.

[2] The "micro" form is described, but the description applies with slight changes to the larger form.

[3] Hempel, German chemist, especially known for his work in gas analysis.

[4] For reagents see Appendix.

[5] Marks at 11.12 and 2.78 mls. are etched upon the sides of the burette in many cases, so that one may fill the burette to the 11.12 mark with nitrite solution and to the 2.78 mark with acetic acid. These amounts represent the stoichiochemical requirements for the interaction of the two reagents.

[6] Reaction II, above, in text.

e being open; the gas forces the liquid into *c*. If the solution is warm, the reaction progresses very rapidly, but it is usual to accelerate it by means of a motor working through an eccentric *k*. When the liquid accumulating in *c* reaches the upper line etched on the burette *c*,

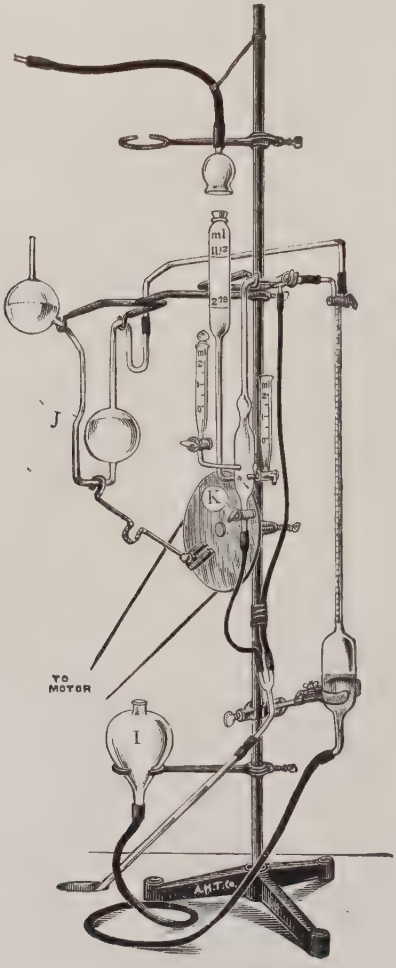


Fig. 113.—Van Slyke gasometric amino-nitrogen apparatus.

stop the motor, open *d*, and let the liquid in *c* force the mixture of gases[1] into the sink through *d*. Close *d* and repeat once again. At the last discharge of the gases, close *d* and allow the liquid to rise in *c* until about 4 mls. of liquid are left in *b*.[2] Close *e*, arrange *d* so that

[1] Including the air which must be removed completely from the whole of the apparatus.

[2] Indicated by a horizontal line etched on the glass.

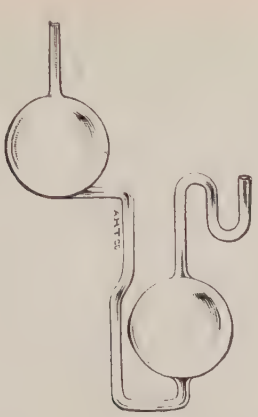


Fig. 114.—Hempel gas pipette for use with the Van Slyke gasometric method for amino-acids. The left-hand bulb serves as a reservoir for the alkaline permanganate which comes into contact with the gas from the deaminization in the bulb to the right.

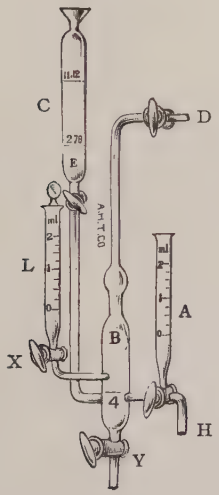


Fig. 115.—Details of the Van Slyke gasometric apparatus for amino-acids. The parts from left to right are: Burette for holding anti-foaming mixture; burette for holding reagents (HNO_2 and CH_3COOH); deaminization bulb; burette for holding unknown being analyzed. (See Figs. 113, 114, 116.)

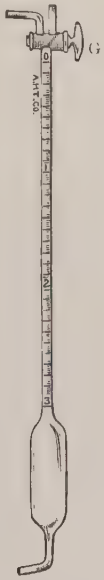


Fig. 116.—The gas burette of the Van Slyke gasometric method for amino-acids.

communication is made to the nitrometer *f*, and open *g* accordingly. Place the level *i* below the bottom of *f*. You have now obtained a supply of nitrous acid in *b* and are ready to add the unknown solution. Deaminization of the amino-acids: This is done by filling the burette *a* with the casein solution and adjusting the meniscus of the liquid to read at 2 mls. by means of the two-way cock *h*, draining the excess fluid into the sink. Now turn *h* so that you may deliver 2 mls. of the casein solution to the deaminizing bulb, *b*. Add a drop or two of anti-foaming solution from *l* and note the time and the room temperature. Start the motor and let run according to the following time limits:

	Minutes.
Temperature 15° to 20° C.	5
Temperature 20° to 25° C.	3
Temperature 25° to 30° C.	2.5

At the end of the allotted time stop the motor, open *e* cautiously, and let the fluid run into *b*[1] and into the communication tubes as far as the cock *g* to insure that all the gas is obtained for analysis. Close *g*, open *d* to the outside, and wash out the deaminizing bulb, *b*, through *c*; the final washings accumulated in *b* are removed by opening *y*. Now raise the level, *i*, and open the two-way cock *g* so that the gas flows into the Hempel pipette, *j*, allowing the water from the nitrometer, *f*, to go as far as the opening into the first of the two bulbs of the Hempel pipette. (3) Absorption of the extra gases and the reading of the column of nitrogen gas: The wire passing from the eccentric wheel *k* is now attached to the Hempel apparatus and by slow[2] motion the gas and the liquids in this part of the apparatus are mixed. After running for about two minutes, stop the motor, lower *i*, open *g* for communication with *f*, and let the residual gas, which is pure nitrogen, pass into *f*, stopping the column of alkaline permanganate at *g*. Bring the level of the water in *i* in the plane of the meniscus of the water in *f*, take the reading, and enter it into your note-book. Clean the apparatus by raising *i* and washing out the gas through *d*. Then let water drive the alkaline permanganate out of the tube connecting with the pipette. You are now ready for another determination. Proceed with the calculation.

Calculation: Since the weight of 1 ml. of nitrogen gas under standard conditions[3] is 0.0012505 g., or 1.2505 mg. we could deter-

[1] Watch the amount of liquid in *c* to insure that it does not run out, thus admitting air. Supplement with freshly distilled water if necessary.

[2] Not over twice per second of time.

[3] 760 mm. mercury pressure (sea-level reading) and 0° C.

mine the weight of nitrogen in the number of mls. found in the above Exercise, if we had this volume expressed in standard pressure and temperature. The table of nitrogen over water[1] gives the weight of

Fig. 117.—Barometer for use in determining the atmospheric pressure in gas analysis. Before reading, the ivory tip in the cistern (below) must be brought into immediate contact with the mercury by turning the milled head. Then the vernier (top) is moved into place by the milled head two-thirds up the tube on the right.

Fig. 118.—Barometer syphon-type. In reading the instrument subtract the reading made on the lower arm from that of the upper arm.

amino-nitrogen[2] in a ml. of gas for various temperatures and pressures. To determine the weight of nitrogen in our sample, enter the table with the data (temperature and pressure) taken at the end of the

[1] Appendix.

[2] The usual tables are for a molecule of nitrogen, N_2; the figures in the amino-nitrogen table are half those of the usual table (as given, for example, in Gattermann's Practical Organic Chemistry. The Macmillan Co.), for only half the N comes from the amino-acid, the other half from the nitrous acid (see Equation I, page 294).

experiment and locate the corresponding factor; multiply this factor by the nitrometer reading made above. The result is the weight of nitrogen gas derived from the volume of amino-acid solution taken for analysis at the beginning of the exercise.[1] If percentage is desired, multiply the result, converted to one ml.,[2] by 100.[3]

The fundamental equation whence the above calculation is derived depends upon the two laws expressing the relation between changes of temperature and of pressure, namely, the Law of Charles and that of Boyle, respectively. Charles' Law predicts: $\frac{V_1}{V_2}=\frac{T_1}{T_2}$, that is, the volume of a gas varies directly with the temperature. The Law of Boyle predicts that $\frac{V_1}{V_2}=\frac{P_2}{P_1}$, or that the volume of a gas varies inversely with the pressure. Combining these we have:

$$\frac{V_1P_1}{T_1} = \frac{V_2P_2}{T_2}$$

and since density and volume are reciprocals, transposing, we have:

$$w = \frac{V_1P_1}{P_2T_2T_1}$$

For additional methods of determining amino-nitrogen see page 743 and Chapter XVI.

PARTIAL SUMMARY FOR PROTIDS

1. Protids are nitrogenous substances which serve a double rôle in the economy of man: (*a*) To restore tissues, and (*b*) To contribute energy in the same manner that glucids furnish energy.

[1] Two mls. were used in this exercise, but other amounts (1, 5, 10, etc.) may be used.

[2] That is, divide by 2, if 2 mls. were used, as in the experiment; by 5, if 5 mls. were used, etc.

[3] The table in the Appendix is derived from the expression:

$$w = \frac{v \times b \times 1.2505}{760\,(1 + 0.00367 \times t)}$$

where:

w = weight of nitrogen sought.
v = observed volume of gas read on the nitrometer.
b = barometer reading taken at end of determination.
1.2505 (mg.) = weight of 1 ml. of nitrogen gas at 760 mms. Hg and 0° C.
760 = standard sea-level pressure (one atmosphere).
0.00367 = the decimal of the fraction $\frac{1}{273}$, the increase in volume of a gas for each degree rise in temperature on the Centigrade scale.
t = observed temperature on the Centigrade scale.

Physical Structure:

2. They are colloidal in nature and have the properties of substances in that state, *i.e.*, to be precipitated and coagulated, to undergo hydrostatic and electrical changes, etc.

3. They react with both acids and bases and are, therefore, typically amphoteric. Certain reagents capable of uniting with substances of either acid or basic nature are capable of causing the precipitation of protids.

Chemical Structure:

4. The chemical structure of protids is somewhat similar to that of glucids and fats, that is, protid is resolvable into certain units, condensed together by the elimination of a molecule of water; during digestion (hydrolysis) water is replaced in the molecule and the several units (amino-acids) are separated into individual molecules.

5. The difference in manner of linkage of the units in glucids and in protids is as follows: In the glucids, the linkage occurs at the oxygen radicle, whereas in the protids, the attachment is with the nitrogen radicle.

6. The criterion of a true protid is its ability to give the biuret reaction.[1] To give this reaction, three or more amino-acids must be linked together, so that there are at least two biuret groups (HNCO) or equivalent.

7. There are about 20 of the amino-acids in most protids, although certain ones contain much fewer.

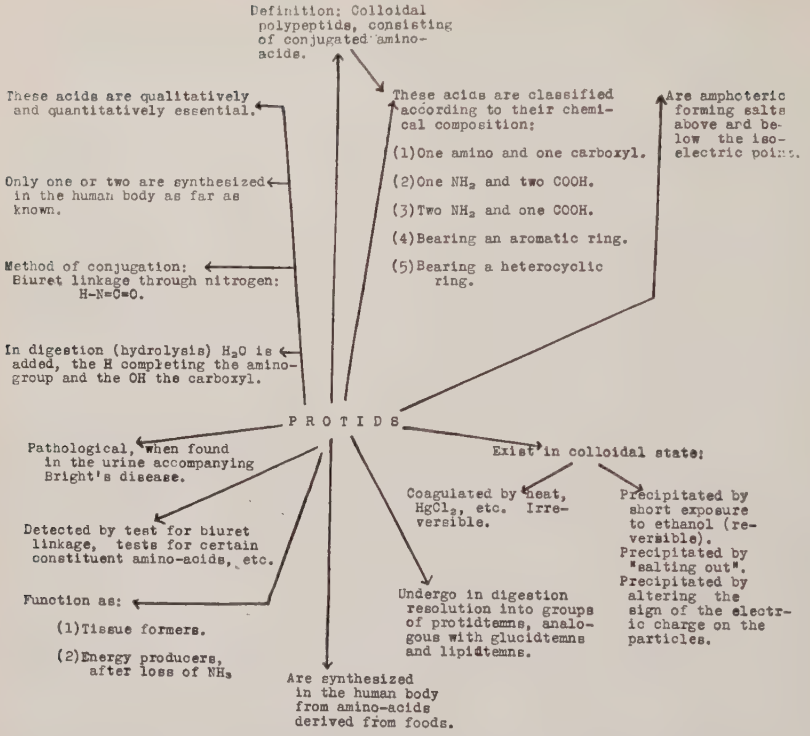
8. Each amino-acid has definite characteristics and serves a given purpose. Except in rare instances one amino-acid cannot replace another in the work of the cell.

9. Destruction of amino-acids may take place in several different ways: (*a*) The acid may become deaminized, the ammonia passing off in the urine as ammonia or conjugated with cyanic acid as urea. If this occur, the residue will become a glucid and share the ability of such substances to become oxidized and to furnish energy. (*b*) It may become decarboxylated and form amins which are toxic. These in turn become detoxicated and excreted as conjugates with other substances, like derivatives of glucose, or salts of sulphuric acid.

10. Comparison of the total nitrogen with the amino-nitrogen of a given protid shows that there is but little of the latter in the protid. However, after hydrolysis, the amount of amino-nitrogen greatly increases, indicating that the amino-acids are linked together in such

[1] In addition to this criterion, the substance must be in a colloidal state, for otherwise a tripeptid could be called a protid, which it is not.

GRAPHIC SUMMARY—PART ONE


Definition: Colloidal polypeptids, consisting of conjugated amino-acids.
These acids are qualitatively and quantitatively essential.
Only one or two are synthesized in the human body as far as known.
Method of conjugation: Biuret linkage through nitrogen: H-N=C=O.
In digestion (hydrolysis) H_2O is added, the H completing the amino-group and the OH the carboxyl.
These acids are classified according to their chemical composition:
(1)One amino and one carboxyl.
(2)One NH_2 and two COOH.
(3)Two NH_2 and one COOH.
(4)Bearing an aromatic ring.
(5)Bearing a heterocyclic ring.
Are amphoteric forming salts above and below the isoelectric point.
PROTIDS
Pathological, when found in the urine accompanying Bright's disease.
Detected by test for biuret linkage, tests for certain constituent amino-acids, etc.
Function as:
(1)Tissue formers.
(2)Energy producers, after loss of NH_3
Are synthesized in the human body from amino-acids derived from foods.
Undergo in digestion resolution into groups of protidtemns, analogous with glucidtemns and lipidtemns.
Exist in colloidal state:
Coagulated by heat, $HgCl_2$, etc. Irreversible.
Precipitated by short exposure to ethanol (reversible).
Precipitated by "salting out".
Precipitated by altering the sign of the electric charge on the particles.

a way as to destroy the amino-radicle, NH_2. Amino-acids are, therefore, linked into the protid molecule by imino-radicles, NH. In this way great complex chains of amino-acids are formed, which are the protids. The difference in kind of protid depends upon the number and kinds of amino-acids condensed into the molecule of protid.

THE CLASSIFICATION OF THE PROTIDS

No satisfactory scheme has been suggested for the chemical classification of protids. The method of classification in use at the present time is based largely upon the physical characteristics of the protids—solubility, etc.[1]

I. Simple Protids.—Hydrolysis of such protid does not produce another protid belonging to this group. On the contrary, the protid may be destroyed by hydrolyzing agents.

[1] An extensive examination of protids from the standpoint of a physico-chemical method of classification has been conducted by Cohn (E. J. Department of Physiology, Harvard) included in reports to the American Society of Biological Chemists. See Jour. Biol. Chem. for the years 1918 ff. Also Physiol. Revs., vol. 5. page 349, 1925.

A. Not Coagulated by Heat in Acid Solution.

1. *Soluble in Certain Reagents in the Cold.* Animal protids.

(*a*) *Protamins.*—Soluble in ammonium hydroxid solution. The reaction is alkaline to red litmus paper.

(*b*) *Histons.*—Not soluble in ammonium hydroxid solution, but in water and in dilute mineral acids. While alkaline, red litmus is not turned blue.

2. *Insoluble in Cold Water and in Many Cold Reagents.*—Animal and plant protids.

(*a*) *Scleroprotids.*—Insoluble in all regents in the cold, but when boiled with strong mineral acids or alkali, they dissolve, being changed to other substances. Animal protids, secretion products of cells.

(*b*) *Prolamins.*—Insoluble in water, but soluble in alcohol of 75 per cent. concentration. Plant protids. Also called gliadins. Always associated with glutelins (page 316).

B. Heat Coagulable.

1. *Insoluble in water.*

(*a*) *Glutelins.*—Immediately soluble in dilute mineral acids and in dilute solutions of strong[1] alkali. Plant protids.

(*b*) *Globulins.*—Slowly soluble in dilute acids and only with the aid of heat. Animal protids.[2]

2. *Soluble in water.*

Albumins.

II. Conjugated Protids.—On hydrolysis, protid and also a "prosthetic group" consisting of a non-protid part are cleaved from the molecule; the protid is a simple one belonging to one of the groups under (I).

A. On Strong Hydrolysis No Phosphoric Acid Is Split from the Molecule.

1. *Glucoprotids.*—A reducing glucid is separated from the molecule on hydrolysis. On account of such glucoprotids as glucosamin (page 354) and galactosamin in many protids, they give a Molisch Reaction (page 171).

2. *Chromoprotids.*—No reducing substance is cleaved during hydrolysis. The prosthetic substance is a colored radicle or compound.

[1] That is, highly dissociating alkali, like NaOH, KOH, etc.

[2] The so-called plant "globulins" belong in another group, owing to their ready ability to become crystallized. In the present scheme they are included in the Globulins of animal origin in order not to complicate the scheme of classification.

B. On Hydrolysis Phosphoric Acid Is Set Free from the Molecule.

1. *Nucleoprotids.*—An ammoniacal solution of the completely hydrolyzed substance will, when treated with silver nitrate solution, yield a brownish or whitish precipitate of purin silver nitrate.

2. *Phosphoprotids.*—No purins occur in the molecule.

CHARACTERISTICS AND DISTRIBUTION OF PROTIDS

The Molecular Weight of Protids.—It has long been known that the molecular dimensions of the protids must be large because of their inability to pass through certain natural and artificial membranes. Many attempts have been made to estimate the molecular weights of the protids by measurements of their osmotic pressure. The earlier efforts were not successful owing to the failure of the investigators to obtain protids free from inorganic constituents. The presence of inorganic ions which can pass through the membranes causes disturbances in the membrane equilibria that have been studied extensively by Donnan (page 107), Jacques Loeb (page 107) and others. The increased osmotic pressure arising from these impurities led to the assumptions for the molecular weights which were much too low.

Sørensen applied corrections to his data to eliminate these errors and obtained figures for the molecular weight of egg-albumin which were very closely in agreement with values obtained by other methods. It is possible to grade the protids with respect to the size of their molecules by the use of membranes of different permeabilities. In this manner, Bechold[1] has arranged the following protids in the order of the increasing size of their molecules: Serum albumin, hemoglobin, gelatin and casein. The best means of determining the minimal molecular weights of a protid depends on the determinations of accurate values for one or more of its constituent atoms or molecules, of which the atomic or molecular weight is known. The minimal molecular weight is then obtained from the following formula:

$$\text{Minimal molecular weight} = \frac{\text{Molecular weight of constituent times 100}}{\text{Per cent. of constituent of protid}}.$$

Cohn[2] and his collaborators have utilized the data obtained from dialysis experiments, from physicochemical measurements of their equivalent combining weights and from analytical determinations of

[1] Bechold, Hans, German physical-chemist, contemporary.

[2] Cohn, E. J., Hendry, J. L., and Prentiss, A. M. (Harvard Medical School, Boston) Papers in the Journ. Biol. Chem., vols. since 1924 and in the Journ. Gen. Physiol., since 1922. See also Physiol. Revs., vol. 5, page 349, 1925.

their elementary and amino-acid compositions for the estimation of the probable molecular weights of a number of protids. The values of the molecular weight of egg-albumin calculated from the osmotic pressure measurements of Sørensen, the centrifugal sedimentation equilibrium of Svedberg[1] and the work of Cohn have shown excellent agreement, as follows:

MINIMAL MOLECULAR WEIGHTS OF EGG-ALBUMIN

Method.	Amt. of constituent.	Weight for one g. mol. of constituent.	Assumed number of atoms of molecules.	Minimal molecular weight.
Osmotic pressure[1]	.....	.....	..	34,000
Centrifuge sedimentation[2]	.....	.....	..	34,500 cir.
Cystin content[3]	0.81	29.654	1	29,654
Tryptophan content[4]	1.23	16.593	2	33,186
Tyrosin content[4]	4.20	4.312	8	34,496
Sulphide sulphur[5]	0.491	6.532	5	32,660
Sulphur[6]	1.616	1.984	17	33,728
Maximal base-combining capacity[6]	....	1.250	27	33,750

[1] Sørensen, S. P. L., Comptes rendus trav. Lab. Carlsberg (Copenhagen) vol. 12, page 262, 1915–17.

[2] Svedberg, T., and Nichols, J. B., Journ. Amer. Chem. Soc., vol. 48, page 3081, 1926.

[3] Looney, J. M., Journ. Biol. Chem., vol. 69, 519, 1926.

[4] Folin, O. and Looney, J. M., Journ. Biol. Chem., vol. 51, page 421, 1922.

[5] Osborne, T. B., Journ. Amer. Chem. Soc., vol. 24, page 140, 1902.

[6] Cohn, E. J., Hendry, L. J. and Prentiss, A. M., Journ. Biol. Chem., vol. 63, page 721, 1925.

The most probable values for the molecular weights of various protids are as follows: (Arranged in order of magnitude)

Protid.	Molecular weight.
Bence-Jones protid	24,500
Edestin	28,000
Egg-albumin	33,800
Fibrin; glutenin	36,000
Serum albumin	45,000
Hemoglobin, horse, ox	66,800
Gliadin	72,900
Serum globulin	81,000
Hemocyanin (blood pigment of king-crab) and of octopus	90,800
Zein	96,200
Gelatin	150,125
Casein	192,000

[1] Svedberg, T., (Danish physical chemist), Nobel-prizeman 1926. See: Svedberg, T. Colloid Chemistry., New York, Chemical Catalog Co., 1924.

Simple Protids

The Protamins.—The amino-acid content of some of the principal protamins is given in the table[1] shown below. The table reveals the preponderance of diamino-acids, arginin, lysin, and histidin. It is this property which renders the protamins capable of uniting with acids, like the nucleic acid of the nucleus to form nucleoprotid. Protamins occur in all nuclei, and for the purpose of quantitative study spermatozoa have been used in which the nucleus is compacted into the "head of the spermatozoön, the cytoplasmic

	Protamins.			Histon.	
	Salmin (Salmon sperm).	Sturin (Sturgeon).	Clupein (Herring).	Globin (Dog's blood).	Thymus Histone (beef).
Glycin					
Alanin	....	....	Present.	3.0	0.5
Valin	4.3	....	Present.	1.0	3.5
Leucin	....	....	....	17.5	
Isoleucin	....	....	.....	....	11.8
Phenylalanin	....	....	....	5.0	
Tyrosin	....	....	....	....	2.2
Serin	7.8	....	Present.	....	5.2
Cystin					
Prolin	11.0	....	Present.	4.5	1.5
Oxyprolin					
Aspartic acid	....	....	....	2.5	
Glutamic acid	....	....	....	1.2	0.5
Tryptophan					
Arginin	87.4	58.2	82.2	....	15.5
Lysin	....	12.0	....	....	6.9
Histidin	....	12.9	....	....	1.5

"tail" being separated by mechanical means. Pure solutions of nucleoprotids have been obtained in this manner. Protamins are characteristic of unripe sex cells like spermatogonia. Although protamins are usually found united with nucleic acid to form the salt, nucleoprotid, they also occur fixed to other protids. In the test-tube they are precipitated when brought into contact with other protids.[2] They are precipitated from their solutions by salts and by acids. Protamins will be discussed farther in connection with nucleoprotids (page 327).

[1] The table includes, also, histons, to conserve space.

[2] And protid derivatives down to, but exclusive of, the secondary albumoses.

Histons are likewise basic and some are more basic than others.[1] They occur in nucleoprotid, like the protamins, but are also found in certain conjugated protids, as, for example, in the protid part of the chromoprotid, hemoglobin. Here the globin is an histon. In the nucleoprotid there is reason to believe that the histon becomes converted into protamin under certain conditions. Examination of the table[2] shows the considerable difference in the content of amino-acids in the various histons; this content varies with the degree of basicity. Histons are characteristic of ripe sex-cells (cf. protamin).

Scleroprotids.[3]—This is an extensive group which formerly bore the name "albuminoids," since it was believed that the substances were modified protids such as are found in egg-white and in blood-plasma. Scleroprotids occur in the exo-skeleton, or protective coverings of the body (hair, nails, cornea of the eye, etc.) as the keratins, and, being resistant to most reagents, are able to a certain extent to protect the underlying and more delicate parts from injury. Connective tissue also contains members of this group, as, for instance, the elastin of the ligamentum nuchæ;[4] collagen, the precursor of gelatin; the "albumoid" of the crystalline lens of the eye, that of cartilage and of bone, the last being known particularly as osseo-albumoid. Keratin contains sulphur; elastin, collagen and osseo-albumoid do not. Reticulin is a member of this group. It is a network underlying the cellular composition of many tissues.

Three scleroprotids (keratin, elastin and collagen) exhibiting characteristic reactions of the group will be presented for practical study.

EXERCISE 39.—*Keratin of Hair:*

(1) Prove that human hair[5] is insoluble in the following reagents:

(*a*) Water.

(*b*) Cold, concentrated H_2SO_4

(*c*) Cold 40 per cent. NaOH solution.

(*d*) Ethanol, 95 per cent.

(*e*) Ethyl ether.

[1] By electrometric titration of thymus histon, Felix (Munich) found that 20 out of every 100 atoms of the histon were freely basic and 12 freely acid.

[2] Page 306.

[3] A division into (1) Sulphur bearing scleroprotids, example keratin and (2) non-sulphur bearing scleroprotids, example gelatin may be made, but none of these groups is a scientific entity.

[4] The common tendon of the trapezius muscle in the neck.

[5] Collected from barber-shops, previously subjected to extraction with alcohol and with ether to remove the fat and grease, then washed with water and dried.

(2) Prove that keratin is soluble in:

(*a*) Hot (boiling) 10 per cent. H_2SO_4 solution.

(*b*) Hot (boiling) 10 per cent. NaOH solution.

(*c*) Alkali sulphide; this solution is used in leather industry in removing hair from hides.

(3) Place a small amount of dry hair in the bottom of a test-tube and heat gently over a free flame. Note the odor of H_2S.[1] The cystin sulphur is known at times as "lead-blackening" sulphur. In the following experiment, which involves a test for the unoxidized, or cystin sulphur, note the blackening of the lead solution:

(4) Test for unoxidized, cystin, mercaptan, or loosely-combined sulphur:[2] Use the egg-white, making a mixture of one volume of the egg-white with one volume of 10 per cent. KOH solution. Add 2 drops of lead acetate; boil. The solution turns brownish to black. A mercaptan odor is given off on neutralizing with a mineral acid. Repeat the above experiment using hair which has been boiled with 10 per cent. caustic alkali (NaOH or KOH).

EXERCISE 40.—*Elastin from the Ligamentum Nuchæ:*[3]

(1) Test as above with the different reagents.

(2) Note its solubility as above.

(3) Prove that cystin is lacking in the molecule of this protid by means of the heating test as in Number 3, above.[4]

(4) Perform the unoxidized sulphur test. It should be negative.

EXERCISE 41.—*Collagen from Cartilage:*[5]

(1) Note the color of the material.

[1] The amount of sulphur in human hair is 15 per cent., corresponding to the percentage of the amino-acid cystin which is present to about that amount, compared to the total amino-acid content.

[2] Compare the statement made on page 234 concerning the impossibility of passing from alkali albuminate, through neutrality, to acid albuminate and of having the same protid as before. Sulphur is split off as demonstrated by the foregoing Exercise. The Exercise also gives a simple test for the kind of sulphur cleaved from the molecule of protid.

[3] Previous treatment of the ligament: Cut the material into small blocks of about 1 cc. This will save time in the laboratory. Wash the pieces. Transfer to a beaker containing half-saturated lime-water; this serves to remove glucoprotid. Wash and place in a beaker with three times its volume of 10 per cent. acetic acid. Boil. Wash with cold water and place in 5 per cent. HCl solution, leaving it one hour. Dry by washing with ethanol and then with ether and dry in a desiccator, or in the open air if protected from dust. The tests are best made on pulverized material.

[4] Exercise 39.

[5] Collagen should be obtained from the tendo achillis. The tendon is cut into small pieces and placed in a trypsin solution to remove protids. The trypsin solution may be

(2) Prove that collagen swells when left in contact with water, dilute acids, or dilute alkali.

(3) Prove that collagen dissolves in cold concentrated caustic alkalies such as NaOH or KOH; that it does not do so in strong solutions of Na_2CO_3.

(4) Place a small piece in water and bring to 70° C. Keep at this temperature for one-half hour and then cool. Transfer to a tryptic digest (page 238). Note that the collagen dissolves. Compare with the statement in the footnote[1] regarding the method of preparation for class-work. Collagen does not digest as such in trypsin solution unless collagen has been previously kept at 70° C. for some time, when it becomes converted into gelatin. Note the odor of ammonia during the process of conversion.

(5) Treat collagen with tannic acid solution. A solid leather is produced.

(6) Gelatin. This is the first hydrolysis product of collagen. Using the commercial material, perform the following tests:

(*a*) Prove the absence of cystin in the protid by the test given on page 253.

(*b*) Note the hydrophilous character of the gelatin (page 128). Is the condition reversible? Frequent solution, especially involving heat, destroys gelatin. A marked difference in solubility exists between purified and crude gelatin.

(*c*) Prove that it is slightly soluble in dilute alkali.

(*d*) Prove its insolubility in 95 per cent. ethanol.

(*e*) Prove that it is not precipitated by strong solutions of highly dissociating ("strong") acids like concentrated H_2SO_4, in the cold.

(*f*) The following tests, while not specific, serve to aid in the detection of gelatin solutions (page 109):

(1) Tannic acid precipitates gelatin, the precipitate being massive.

(2) Gelatin is precipitated by mercuric chlorid in the presence of hydrochloric acid.

(3) Gelatin is precipitated by saturating its solution with magnesium sulphate and by adding one volume of saturated solution of ammonium sulphate to one volume of the gelatin solution. It is

made from a weak sodium carbonate solution into which some commercial pancreatin is placed. It is preferable to grind a piece of the duodenum with the pancreatic tissue. Care must be taken to obtain pancreas, as thymus gland is often sold as sweetbread at the market. "Belly-sweetbread" is the language used by the butcher to designate pancreas. The cartilage must be washed with cold water before use in the experiments. Collagen is the "white fibres" of the histologist.

[1] See footnote 5, page 308.

likewise precipitated by saturating the solution with sodium chlorid and then acidulating with a few drops of a mineral acid.

(4) To distinguish gelatin from its hydrolysis products: Add one volume of Esbach's picric-citric acid solution:[1] Gelatin is precipitated while the products, like pepton, are not.

The amino-acid content of gelatin: Gelatin is usually considered an incomplete protid, *i. e.*, it does not contain a complement of amino-acids.[2] Those lacking are very important ones.[3] By virtue of this fact, gelatin cannot be used to restore the nitrogen loss in the urine. Hence an animal fed exclusively on gelatin as its protid food does not develop normally and, after a while, succumbs. The reason for this is made evident by an examination of the amino-acid content of gelatin which is given in the following table. By the butyl-alcohol method of analysis of Dakin, 91.3 per cent. of the total amino-acid nitrogen content of gelatin is accounted for:

	Casein.	Gelatin.	Zein.	Lactal-bumin.	Gliadin.	Edestin.	Silk fibroin.
Glycine	0.45	25.5	0.0	0.37	0.0	3.80	40.5
Alanine	1.85	8.7	3.8	2.41	2.00	3.60	25.0
Valine	7.93	0.0	1.9	3.30	3.34	6.20	0.0
Leucine	9.7	7.1	25.0	14.03	6.62	20.9	2.5
Phenylalanine	3.88	1.4	7.6	1.25	2.35	3.09	1.5
Tyrosine	5.36	0.0	5.66	1.95	3.04	4.58	11.0
Serine	0.5	0.4	1.0	1.76	0.20	0.33	1.8
Cystine	0.25	0.16	0.75	4.1	2.40	0.84	0.0
Proline	8.7	9.5	9.0	3.76	13.22	4.10	1.0
Hydroxyproline	0.23	14.1	0.0	0.00	?	2.00	0.0
Aspartic Acid	4.1	3.4	1.8	9.30	0.58	4.50	0.0
Glutamic Acid	21.77	5.8	31.3	12.89	43.66	18.74	0.0
Hydroxyglutamic Acid	10.5	...	2.5	10.00	2.40	?	0.0
Tryptophane	1.6	0.0	0.0	2.40	1.10	1.52	0.0
Arginine	3.81	8.22	1.82	3.47	3.2	14.17	1.5
Lysine	8.38	5.92	0.0	9.87	0.63	1.65	0.85
Histidine	2.5	2.94	0.82	2.61	1.49	2.19	0.75
Ammonia	1.61	0.49	3.6	1.31	5.22	2.28	0.0
Total	93.12	93.63	96.55	84.78	91.45	94.49	86.40

These results are tabulated from the values published by Dakin; Foreman; Jones, Gersdorff and Moeller; Osborne; Folin and Looney; May and Rose; and Looney.

The following amino-acids are either absent from the molecule of gelatin, or present in such minute amounts that they are inadequate:

(1) Isoleucin.

(2) Tyrosin.

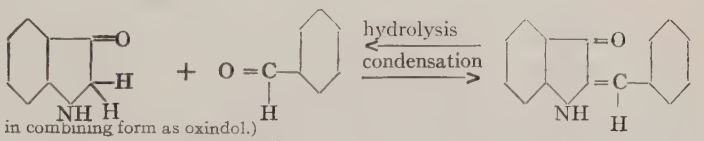
(3) Cystin.

[1] Appendix.

[2] Or perhaps further work will show that it is not the entire lack of amino-acids, but insufficient quantity of these substances; compare the statement below for tryptophan.

[3] Gelatin has been crystallized (see Bradford in "Nature," vol. 111, page 200, 1923, but Knaggs and Schryver (page 346) do not believe that gelatin is a chemical entity.

Tryptophan is usually said to be absent, but Kraus[1] has been able to identify it by means of the bromin reaction. Of the three, the last two are especially important. We have found that gelatin gives a negative reaction to the test for cystin.[2] Millon's tyrosin test[3] is also negative, proving that tyrosin is absent. The process of gelatin production from collagen may be responsible for the negative reaction given by gelatin to tests which depend upon the presence of the indol ring;[4] for it is known that hydrolyses and other chemical reactions in some unknown manner render tryptophan refractory to the tests. The principle of these various "tryptophan" tests is the same as that encountered in the test for indican,[5] in which indigo is produced by the conjugation of two molecules of substances containing the indol ring. Many other colored compounds of indol occur and are used commercially. In the various tests which have been devised for tryptophan, an aldehyde, like formaldehyde or glyoxaldehyde, is caused to condense with tryptophan to form one of the group of condensation products known to the organic chemist as indogenides.[6] As an example of such condensations the following reaction between indoxyl and benzaldehyde, as given by an early student of the pure chemistry of these groups, is cited:



(Written in combining form as oxindol.)

Indoxyl. Benzaldehyde. Indogenide of benzaldehyd (colored).

The substance produced is first yellow, but on standing it becomes blue by oxidation. If, however, it is left standing for some time, it becomes hydrolyzed to the colorless indoxyl compound again. We shall apply the principal tests to egg-white which gives a positive reaction, and then we shall determine the absence or presence of a very minute amount of tryptophan in gelatin.

EXERCISE 42.—On a 1 per cent. solution of egg-white apply the following tests:

[1] Kraus, Ida (University of Chicago). See Jour. Biol. Chem., vol. 63, p. 178, 1925.
[2] Page 309.
[3] Page 241.
[4] Page 312. Tryptophan bears the indol ring (page 277).
[5] Page 758.
[6] Pronounced "in-dog'en-ides."

(1) *Adamkievick's Reaction.*[1]—To 1 volume of egg-white solution add 2 volumes of glacial acetic acid[2] and mix; then layer with one volume of concentrated, pure, colorless sulphuric acid by letting the acid run down the inner sides of the inclined test-tube. At the junction of the concentrated acid and the mixture of protid and acetic acid, note the reddish-violet zone, which may be accentuated by letting the preparation stand for a short time, or by gently rotating the tube between the palms of the hands. The zone of color gradually spreads throughout the preparation.

(2) *Hopkins-Cole Reaction.*[3]—Use the reagent known as "reduced oxalic acid"[4] which is a solution of glyoxalic acid itself: To one volume of the protid solution add 1 volume of the reagent. Mix. Layer as in (1) with concentrated sulphuric acid. In this reaction, as in other similar methods, chlorids, nitrates, and chlorates, especially when present in large amounts, interfere with the reaction. The color is similar to that obtained in the preceding method (1).

(3) *Rosenheim's Test, Modified by Acree.*[5]—Formaldehyde in very dilute solution (1:250, aqueous) is used as the aldehyde: To 1 volume of the protid solution, add, while shaking the test-tube, 1 drop of the reagent. Dilute with 1 volume of water and layer as before with sulphuric acid. The addition of a drop of ferric chlorid solution will accentuate the color and increase the rapidity of its appearance. A solution of formaldehyde 1:10,000 will give the reaction if a protid containing more than a mere trace of tryptophan is used.

(4) *Liebermann's*[6] *Reaction.*—This reaction likewise depends upon the condensing action of an aldehyde or other carbonyl[7] group, which is furnished in the form of alcohol containing an aldehyde or anhy-

[1] Adamkievick, A., Vienna pathologist, 1850–1921.

[2] The reaction is not by way of the acid, acetic acid, CH_3COOH, but by the impurity, glyoxylic acid, which is contained in all but the better commercial preparations of glacial acetic acid. Glyoxylic acid is $\begin{matrix}CHO\\|\\COOH\end{matrix}$; the aldehyde group is concerned in the condensation.

[3] Hopkins, F. G., Professor, Cambridge University, England; Cole, S. W., Lecturer in the same institution. Both contemporary.

[4] Appendix.

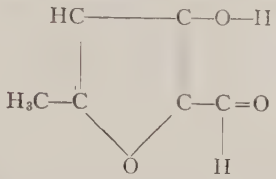
[5] Rosenheim, Th. (contemporary), German physician; Acree, S. F. (contemporary), chemist, formerly of the U. S. Forest Products Laboratory, Madison, Wisconsin, and of Syracuse University.

[6] Liebermann, L. von S. (contemporary), Hungarian physician and biochemist, 1852.

[7] Page 145.

drid;[1] or the aldehyde may be furnished by a glucid containing an available carbonyl group, cane-sugar generally being used. Reaction by alcohol: To 1 volume of ethanol add 1 volume of egg-white and heat. Filter. Wash the residue with ether, scrape off the protid from the filter-paper, suspend it in water, and treat with 1 ml. of concentrated H_2SO_4 Heat. The characteristic color appears.

Reaction by Sucrose: To 5 mls. of egg-white preparation add half a ml. of 1 per cent. cane-sugar solution. Mix. Then layer with concentrated sulphuric acid as before. The color is reddish. Since sucrose itself contains no free carbonyl groups, the reaction is explained by the production of an aldehyde, methylhydroxyfurfuraldehyde:



(5) *Qualitative Test for Tryptophan in a Mixture of Amino-acids, Indol, and Skatol: The Bromin-water Reaction.*—A water solution of tryptophan absorbs bromin readily, developing a pink to a violet color which is soluble in amylol. This color is easily discharged when a slight excess of bromin-water is added. Indol and skatol also absorb bromin under similar conditions; when bromin water is added to a mixture of indol, skatol, and tryptophan there is danger of adding too much bromin and obscuring the test; also, the color extracted by amylol from such a mixture is brownish-red instead of pinkish-violet. This can be overcome by shaking the mixture with toluene and removing indol and skatol.

Procedure.—Shake a neutral solution of amino-acids in which indol and skatol are suspected first with half or an equal volume of toluene; then take 2 mls. of the aqueous solution, add to it 1 ml. of amylol, then very gradually, while shaking drop by drop, freshly prepared bromin water. A pink to a violet color will appear in the amylol layer if tryptophan is present.[2]

Exercise 43.—Using a 2 per cent. gelatin solution, made by dissolving gelatin in boiling water and then cooling, repeat the above tests.

[1] Compare the Liebermann-Burchard test for cholesterol, page 216, in which acetic anhydrid is used similarly as the reagents in the present set of reactions.

[2] Levene, P. A., and Rouiller, C. A. (Rockefeller Institute), Jour. Biol. Chem., vol. 2, p. 481, 1906. Homer, A., Jour. Biol. Chem., vol. 22, p. 374, 1915.

Quantitative Method of Tryptophan Determination in a Mixture of Amino-acids, Indol, and Skatol.[1]—*Principle*—Indol and skatol are soluble in toluene and may be removed from a water solution by toluene extraction. Tryptophan is then separated by mercury precipitation under proper conditions. Chlorides inhibit this precipitation. The optimum color of the tryptophan vanillin-HCl is best developed in the presence of excess mercuric ion; an additional amount of $HgSO_4$ is added to the isolated tryptophan mercury precipitate. Since mercury is a factor in the color development the standard must be treated in the same way as the solution to be tested, *i. e.*, mercury precipitation and further addition of $HgSO_4$ solution is essential. (For reagents see Appendix.)

Procedure.—A. Shake the mixture containing indol, skatol, tryptophan, and other amino-acids in a separatory funnel several times with toluene.

The final toluene phase should give no color with vanillin-HCl.

1. The combined toluene extracts contain the indol and skatol.
2. The aqueous phase contains all of the amino-acids.

B. Determination of tryptophan in the aqueous phase.

1. Take a portion of the aqueous solution and make proper dilution so that a volume of 5 mls. or less shall contain at least 0.25 and not more than 1 mg. of tryptophan and place it in a 15-ml. centrifuge tube.

2. Add thereto 1 ml. of the 50 per cent. H_2SO_4 and 3 mls. of the $HgSO_4$ reagent. Stopper tightly and shake well. Rinse stopper with 5 per cent. H_2SO_4 and make up to 10 or 12 mls. volume.

Let stand for two or more hours, then centrifuge for ten minutes. The tryptophan-mercury precipitate will settle, the clear supernatant liquid is poured off, and the tube allowed to drain for a few minutes.

The precipitate is then washed with 5 per cent. H_2SO_4. It is centrifuged again, decanted, and drained.

3. To the centrifuge tube containing the tryptophan-mercury precipitate add 0.4 ml. of the vanillin solution, 1 ml. of the 2 per cent. $HgSO_4$ solution, and 0.5 to 1 ml. of the concentrated HCl to dissolve the precipitate. This solution is then rinsed into a 50-ml. volumetric flask by using small portions of the concentrated HCl at a time until 15 mls. are used, which is the minimum amount necessary for the reaction.

[1] Kraus' method; see page 311.

Set aside for twenty-four hours, then dilute to 50 mls. with water, and compare with standard prepared in the same way in a colorimeter within several hours.

Prolamins.—If wheat flour is manipulated with a small amount of water,[1] as in the preparation of bread dough, a spongy mass is obtained. If the dough thus obtained is kneaded under running water the starch will be washed out, leaving protid, more or less free from starch. Now if this protid mass is covered with 70 per cent. ethanol and left for twenty-four hours, then filtered, a protid may be detected in the alcoholic filtrate. This is the protid, gliadin, a member of the prolamins, which are alcohol soluble. Using a preparation made as described above,[2] perform the following Exercises:

Exercise 44.—Remove any alcohol which is present by placing the gliadin in an evaporating dish on a water-bath for ten minutes; then transfer the dried gliadin to a mortar and pulverize it. With this pulverized material:

(1) Determine the insolubility of gliadin in water.

(2) Determine its solubility in dilute solutions of weak and strong acids and alkali; also that it is soluble in dilute solutions of salts like NaCl, Na_2CO_3, etc.

(3) Determine its solubility in ethanol of different strength—25 50, 75, 95 per cent., and "absolute."[3]

(4) Prove that gliadin is not coagulated by heat.

Other prolamins, such as zeïn of corn, hordeïn of barley, etc., exist. Gliadin occurs not only in wheat but also in rye. However, in making bread from any grain except wheat, wheat flour is used to supplement the protid, since the physical factor of sponginess possessed by gliadin is not shared by other prolamins to a sufficient extent. Even in rye the gliadin is not present in the amounts required for making good bread from rye flour alone. The amino-acid content of plant protids is given in the table shown on page 310.

It is evident that all the prolamins listed in this table are deficient in some of the important amino-acids, like cystin, and that the amount of tryptophan is small. Attention should be directed to the large content of glutamic acid. Since the average amount of this amino-acid in animal tissues is about one-third of that occurring

[1] The amount of water must be small, or a pasty mass incapable of being worked will be formed.

[2] The residue from this preparation should be saved for the study of the glutelins, page 316.

[3] For tables of alcohol dilution see Appendix.

in these plant protids, the human body must dispose of a large excess of nitrogen when the food contains such materials to any extent.[1] The name "prolamin" is significant of the relatively large amount of prolin.

The amino-acid lysin is absent from these protids.[2] Consequently much experimentation has been done with them to determine whether this amino-acid is essential to growth and maintenance in animals.[3] The effect of feeding an experimental animal with food deficient in lysin is shown in Fig. 166. The normal individual is protected against deficiency diseases due to the absence of certain amino-acids by his general diet. Substances which contain these important constituents of the protids lacking in certain foodstuffs enter the body on an omnivorous diet. The diet of corn bread and milk common among many races illustrates this point. The lysin deficiency of corn protid is counterbalanced by the relatively large percentage of lysin in casein (8.4 per cent.). Moreover, corn itself has another protid which contains lysin (3 per cent.). This protid is a member of the glutelins.

Osborne has found an alcohol-soluble protid in milk. This departs from the usual characteristics of animal protids which are insoluble in alcohol.

Glutelins.—If the biuret test is applied to the residue of wheat flour left after the alcohol-soluble protid, gliadin, has been extracted, it will be found that protid is still present. This protid is glutenin, a member of the glutelins. The group includes the glutenin of wheat, legumin of the pea and bean, phaseolin of the white kidney-bean, amandin of the almond glycinin of the soy-bean, conglutin of the European lupine seeds, avenin of the oat, etc. The glutelins are heat coagulable, thus differing from the prolamins. They are insoluble in water, but are soluble in 0.2 per cent. diluted acids and alkali. With the exception of the so-called "plant globulins," discussed below, they are insoluble in dilute salt solutions. They contain qualitatively and quantitatively amino-acids adequate for human use as food, but cystin is not present, as a rule.[4] Since certain glutelins

[1] Refer to Chapter XII for a discussion of the "physiological economy of nutrition" studied in former years by Chittenden, McCollum, and others.

[2] It is said to occur in very small amounts in wheat gliadin.

[3] This is the work of Osborne and Mendel at New Haven. For an account of this work see the volumes of the Journal of Biological Chemistry from 1910 to 1916. The matter is discussed in more detail in pages 547–549 of this book.

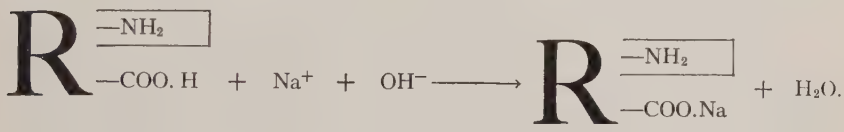
[4] Cystin is present to the extent of 0.3 per cent. in the protid belonging to this series derived from the pine-needle. This food material has been used for supplying deficiency of vitamins likewise.

like legumin exhibit characters somewhat similar to those of the following group, globulins, they are classified as Plant Globulins, which are soluble in dilute salt solutions (legumin soluble in 0.01 per cent. of a saturated $(NH_4)_2SO_4$ solution).

The distinctions between the plant protids concern mainly their iso-electric points.[1] The globulins, while practically amphoteric,[2] are more soluble in alkaline than weak acid solutions because their iso-electric point les on the acid side of the neutral point (pH 5.0±). Alkali suppresses the activity of the basic radicle, NH_2, of the protid, leaving the carboxyl, as we have described earlier in the book[3] free to unite with the alkali to form a salt. This is conventionally represented by Loeb[4] as follows:

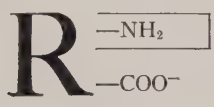
Protid molecule in alkaline medium.

where R is a protid having the free amino- and carboxyl groups, but the former, NH_2, being suppressed in the alkaline medium[5] leaves the carboxyl free to unite with cations in the medium; if the medium is a dilute solution of NaOH, then the salt, sodium protidate, is formed:



Protid in alkaline medium; the protid acts as an anion.

The compound ionizes as



and

Na^+

Protid anion. Sodium cation.

[1] See Table of Isoelectric Points, Appendix.

[2] Page 108.

[3] Page 108.

[4] Page 107.

[5] This is an extension of the great Law of Le Chatelier stated by Deming as: "Systems in equilibrium change only with changing external conditions and then only in a way which tends to restore original conditions." When ions of the same kind are introduced into a solution the mass action is altered and there is a tendency toward less dissociation of the ion involved.

The ions remain in solution in the alkaline medium, but if acid is added to bring the *p*H to 5.5, precipitation occurs. This is the iso-electric point at which the activity of the NH_2 group manifests itself, neutralizing the effect of the carboxyl. The iso-electric points of the plant globulins have not been determined fully, and hence no scientific classification can be given for this group. It is therefore included among the Glutelins.

Glutelins contain proportionately more nitrogen than animal protids, the average being about 21 per cent. The factor used for conversion of nitrogen into protid when dealing with plant protids is 4.78.[1]

Globulins.—These protids, together with the following group of albumins, comprise the "native protids." They occur conspicuously in substances used largely in experimentation and in class demonstrations, such as egg-white and blood-serum. Since in both cases the protids are extracellular substances[2] of the nature of secretions, the question may arise as to whether the native protids are true protoplasm, such as that which exists in the cytoplasm of the cell. The meager studies which have been made upon cells, as such, show without doubt that egg-white and blood-serum or plasma are not typical cellular protids, although they do occur in the cytoplasm of the cell.[3]

The principle of heat coagulation of protids has already been discussed (page 226). Pure crystalline globulin from egg-white can be kept in clear solution for months if the temperature be kept low (2° C.);[4] indeed, the temperature may be raised to 60° C. without causing change; but if the acidity of the solution is now raised to *p*H 4.8 (the iso-electric point) coagulation will occur at once owing to the loss of power on the part of the protid molecule to attract water. If more acid is added, thus decreasing the *p*H, heat coagula-

[1] That is, $\frac{100}{20.9} = 4.78$, expressed as a decimal in place of a common fraction. The corresponding figure for animal protids is 6.25, where the nitrogen occupies 16 per cent. of the protid.

[2] That is, lying outside the cell proper. The blood has been known as a "liquid tissue," the form elements (erythrocytes and leucocytes) being cells suspended in what is virtually intercellular material, the plasma. This intercellular material is comparable with the substances secreted by cells of other tissues and which surround the cells proper, like the cartilage surrounding the hyaline cartilage cells, the bone matrix surrounding the bone-cells, etc. In a similar way, the egg-white is a secretion of the cells of the oviduct.

[3] See Oppenheimer, C., Handbuch der Biochemie, 1st ed., vol. i, Jena, Gustav. Fischer. Also Leathes, J. B. Croonian Lecture, 1923, Lancet (London).

[4] The average ice-box or refrigerator seldom is at a lower temperature than 8° C

tion will occur at a higher temperature,[1] while on acid the side of maximum, *p*H 4.25, heat coagulation will not occur, even if the temperature is raised to 96° C. This point, *p*H 4.25, is the critical *p*H for heat coagulation of egg-white.[2] An alkalinity greater than 4.25 causes, progressively, diminishing coagulation. The effect of a neutral salt, like NaCl, is to inhibit precipitation on coagulation; hence neutral salts cause water to dissolve globulins, the protid salt formed with them is more soluble than "iso-electric" protid. Globulins are insoluble in distilled water, but are soluble in dilute salt solutions. On the other hand, they are precipitated by saturating their solutions with NaCl,[3] or with magnesium sulphate, or half-saturating the solution with ammonium sulphate.[4] These concentrated salts precipitate by absorbing water from the globulin.

We have already studied albumin, one of the typical protids mentioned above in the earlier part of the division on protids.[5] This will suffice for practical experience with the coagulable "native" protids.

Albumins.—The albumins differ from the globulins in that, as a group, their iso-electric points are farther on the acid side of neutrality.[6] Salts tend to diminish ionization and hence to cause precipitation in such solutions. In pure water albumins are soluble and also in low concentrations of salts, a point which is sometimes overlooked in considering the differences between the two. The globulins are precipitated by much lower concentration of salts than albumins. The increase of acidity of an albumin solution permits the use of smaller amounts of salt to cause precipitation,[7] a fact well known to the housewife, who adds a drop of vinegar to the egg she is poaching to cause better coagulation.

The chief chemical differences between albumins and globulins are: (1) The absence of glycin from the molecule of albumin and (2) the greater content of sulphur in albumin. The cystin content of globulin and albumin should be compared in the following table (bloodserum):

[1] Coagulation occurs with *p*H 4.39 at 80° C.

[2] Compare "critical temperature" and "critical pressure" of solutions (Mellor, vol. 1); see citation, page 21.

[3] NaCl is monovalent; $(NH_4)_2SO_4$ is divalent; hence twice as much, roughly, of the NaCl must be used. See page 227.

[4] $(NH_4)_2SO_4$ in 40 per cent. solution at room temperature (20° C.).

[5] Page 226.

[6] *p*H 5.4 to 4.7.

[7] For example, at *p*H 2.0 a half-molecular solution (3 per cent.) of NaCl will cause precipitation, while at *p*H more alkaline than 2.0, even a saturated solution of this salt (36 per cent.) will not cause precipitation.

	Globin of blood.	Globulin of blood.	Albumin of blood.
Glycin	0.0	3.5	0.0
Alanin	1.2	2.2	2.7
Valin	....	Present	?
Leucin	29.0	18.7	20.0
Isoleucin	....	?	?
Phenylalanin	4.20	3.8	3.1
Tyrosin	4.64	6.7	4.63
Serin	.60	?	0.6
Cystin	.75	0.7	2.88
Prolin	2.30	2.8	1.0
Oxyprolin	....	?	?
Aspartic acid	4.40	2.5	3.1
Glutamic acid	1.70	8.5	7.7
Tryptophan	2.61	2.28	1.79
Arginin	8.00	3.95	4.9
Lysin	11.10	8.9	13.2
Histidin	12.70	2.8	3.4

Conjugated Protids

The Glucoprotids.[1]—An example of the members of this group is mucin, which occurs in saliva, connective tissue, intestinal secretions, bile, etc.

Exercise. 45.—Mucin of saliva. Wash the mouth with water. Chew paraffin wax and collect the saliva by expectorating upon the filter-paper fitted to a 250-ml. funnel. Use the filtered saliva for the following Exercise: Place 10 mls. of the filtrate in a beaker and add, drop by drop, glacial acetic acid. The stringy precipitate is mucin. Collect it by means of a small glass stirring-rod. Save the fluid. Divide the mass into two portions and to one add drop by drop 1 per cent. solution sodium carbonate until the mucin is dissolved. Use this material for the tests:

(1) Apply the biuret test to 2 mls. in a test-tube; the reaction is positive and typical, which proves that mucin is a true protid.

(2) Hydrolyze the conjugated protid, mucin, by boiling the remainder of the alkaline solution with 1 drop of concentrated HCl, neutralize to litmus paper with one or more drops of 10 per cent. NaOH solution and make Benedict's qualitative test[2] for reducing substance. Glucose is present and may be identified by means of the osazone.[3]

[1] Called also glycoprotids.
[2] Page 160.
[3] Page 163.

(3) Make Millon's test[1] and Moerner's tyrosin test[2] on the fluid saved after the mucin had been removed (at the beginning of this Exercise). A slightly positive reaction may be obtained, but the Exercise indicates that mucin is the principal protid of saliva.

(4) Using the remainder of the mucin from the beginning of the Exercise, add, drop by drop, 1:1000 solution HCl; note that the mucin dissolves. Mucin is soluble in dilute solutions of alkali and acid.

EXERCISE 46.—Mucin from connective tissue: Secure a piece of tendo achillis from the store-room and cut it into small pieces. Soak these in 0.9 per cent. NaCl solution over night or until the next period and then wash with water in a beaker. Cover the pieces with lime-water and leave again over night, or until the next period. Then add, drop by drop, glacial acetic acid to precipitate mucin as before. Purify the preparation by dissolving the precipitate of mucin in lime-water[3] and leave again over night or until the next period. Wash with cold water, then with alcohol, and use the preparation for the tests given under:

EXERCISE 47.—Try its solubility in 0.1 per cent. HCl and in 0.2 per cent. HCl solutions. Compare page 308, in which keratin from hair is shown to contain a sulphuric acid derivative.

Other linkages of glucid and nitrogenous substances occur in the human body. For instance in the nucleoprotid about to be discussed,[4] a hexose[5] or pentose is present in the molecule. Glucose occurs in "pure" protids, such as egg-white, the composition of which is as follows:[6]

IN ONE HUNDRED PARTS OF EGG-WHITE ARE

Solid matter, 13.3 grams.
Water, 96.7 grams.
Of the solid matter, protid, 12.2 grams per 100 grams solid.
Of the solid matter, glucose, 0.5 grams per 100 grams solid.
Of the solid matter, ash, 0.66 grams per 100 grams solid.
Of the solid matter, fats, etc., trace.
Of the protid, globulin, 6.7 grams per 100 grams solid.
Of the protid, albumin, 83.3 grams per 100 grams solid.
Of the protid, glucoprotid makes up 6.7 grams per 100 grams solid.

[1] Page 241.

[2] Page 241. These tests are for the purpose of demonstrating that mucin is a protid.

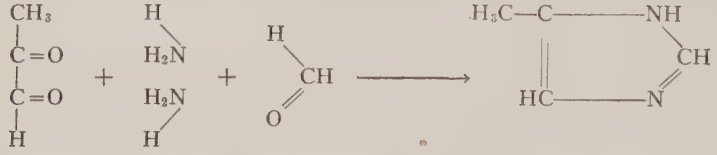
[3] Appendix.

[4] Page 325.

[5] In animals, a pentose in plants.

[6] Plimmer, R. H. A., Practical Organic and Biochemistry, New York, Longmans, Green & Co., 1915, p. 434. Later editions of this valuable book have been issued.

Two types of glucose occur in egg-white: (1) Glucose more or less free, or loosely bound, perhaps as a labile glucoside[1] and (2) bound as glucoprotid, called ovomucin. Glucose may be utilized in man in the synthesis of nucleoprotid or even of the amino-acid histidin and other compounds which contain the iminazol ring.[2] It was shown on page 176 that in both fermentation and glucid metabolism, methylglyoxal may arise from glucose which is cleaved in the middle of the six-carbon chain to make two three-carbon chains. Methylglyoxal then may conjugate with ammonia to form methyl-iminazol as follows:



Methyl-glyoxal. Two ammonias. Formaldehyde. Methyl-iminazol.

An aldehyde similar to formaldehyde is involved which may be supplied by the second (lower) half of the glucose molecule. This configuration occurs in the molecule of the purins which are constituents of the nucleoprotids (page 329).

In the greater number of protids there is a glucid portion or portions. For the detection of this configuration Molisch's reaction is used as described on page 171.

Exercise 48.—Apply the Molisch test to the following protids, all of which give positive reactions: (1) Egg-white; (2) blood fibrin; (3) mucin derived as above; (4) a solution obtained by hydrolyzing the shell of lobster or cray-fish (chitin), the solution being neutralized before testing. Note that the chitin of such animals[3] is different from the keratin of human hair, nails, etc.

Chromoprotids.—Theoretically, many chromoprotids may exist, but the properties of only two or three have been studied. For the medical student hemoglobin is the only one of importance. All vertebrates, including those in which the column is cartilaginous, or in the condition of the embryonic notochord, have hemoglobin. This substance differs in different animals as the exhaustive studies on the crystallography of hemoglobin by Reichert[4] show. Like all

[1] Page 188.

[2] This configuration was discussed on page 272 in connection with histidin.

[3] Chitin is a glucosamin, the glucid portion being a pentose. (See galactosamin, page 354.)

[4] Reichert, E. T. (1855, contemporary), Professor of Physiology, retired (1920), University of Pennsylvania, Philadelphia, at present resident of Florida. See his Monograph on Hemoglobin published by the Carnegie Institution of Washington. The plates were prepared by Brown, formerly of the University of Pennsylvania.

conjugated protids, hemoglobin has two portions: the protid (94 per cent.) and the prosthetic portion (6 per cent.). The differences between chromoprotids are confined to the protid fraction.

Exercise 49. Prepare crystalline hemoglobin as follows:

(1) Rapid method: Centrifuge oxalated blood,[1] discarding the plasma (upper) layer. To the corpuscles (lower layer) add enough distilled water to replace the total volume of whole blood before the plasma was removed. Centrifuge the blood-corpuscle suspension. Decant the supernatant clear, red solution of hemoglobin from the residue (stroma of corpuscles, leucocytes, etc.) and add to it one volume of toluene. Mix well and leave in a cold place over night or until the next period. Long crystals of hemoglobin should be found. Decant the fluid, filtering if necessary, and washing with a very dilute 0.1 per cent. HCl solution.

(2) Preparation of pure hemoglobin by the Heidelberger[2] method: Horse blood[3] is preferable, but dog blood may be used. Defibrinated blood is employed. Secure 30 mls. of the defibrinated blood from the store-room and fill two 15-ml. centrifuge tubes with it. Place the tubes upon the balance and bring them to equilibrium[4] by adding a drop or two of the blood as required. Centrifuge at half speed for two minutes. Open the contact and let the centrifuge come to a stop; then lift the tubes carefully from the cups and by means of a 5-ml. pipette remove the supernatant serum and also the narrow whitish zone of leucocytes and platelets at the junction of the serum and the red corpuscular layer. Discard the serum and white-cell decantation and restore the original volume in the centrifuge tube by adding ice-cold 0.85 per cent. sodium chlorid solution.[5] Centrifuge and again decant; test the decanted portion for protid by boiling a small quantity of it with a drop of acetic acid. If there is still cloudiness, repeat the washing of the corpuscles until only a faint haze is seen. By means of a small amount of distilled water transfer the corpuscles

[1] Page 71.

[2] Heidelberger, M. (Rockefeller Institute, New York, N. Y.). See Jour. Biol. Chem., vol. 53, p. 31, 1922.

[3] It is easy to obtain sufficient horse blood for class work by inserting a fairly strong needle (spinal-fluid needle) into a superficial vein of a horse. The operation involves scarcely as much pain as the bite of a fly, and from 100 to 200 mls. of blood may be withdrawn without difficulty without the use of a syringe. No anticoagulant is used, but the fibrin is removed by whipping the blood collected in a beaker with a test-tube brush. The fibrin should be preserved for Exercise 3, page 434.

[4] In order that the two sides of the centrifuge head be of the same weight and that no eccentric action harm the instrument.

[5] Compare page 122.

after the last decantation to a small Florence flask embedded in chipped ice and pass a stream of carbon dioxid[1] gas from the generator. After the gas has passed for a short time, add 3 mls. of ether, still continuing the passage of the gas. Stir to make a paste and then stopper tightly. Leave until the next period in the ice-box. It is advantageous to shake the corpuscles with air in order to oxygenate the hemoglobin. Centrifuge for two minutes and collect the crystals of hemoglobin resting in the bottom of the centrifuge tube by decanting the two supernatant zones (1) the top layer of cells and (2) a middle layer of clear solution. The crystals may be dried by transferring them with a small amount of ice-water to a Buchner funnel and filtering off the water with suction, using hardened filters to receive the crystals. Transfer some of the crystals to a microscope slide and cover with a cover-glass. Examine and sketch their shape. Save the excess crystals for Exercise 50 below.

This procedure may be shortened by treating defibrinated blood with toluene in the proportion given above for ether, bubbling CO_2 gas through the solution, shaking with air, and leaving in the ice-box until the following period. The resulting crystals will not be typical, but will serve for the study of the globin content. These impure crystals cannot, however, be used for later study of the chemistry of hemoglobin.

Exercise 50.—*Decomposition of Hemoglobin.*—Take 2 mls. of the washed crystals and dilute with one volume of distilled water. Add 1 ml. of concentrated HCl and boil for five minutes. Decant one-half of the solution from the test-tube into another tube and neutralize with a few drops of 10 per cent. NaOH solution, using litmus-paper as indicator. Make Millon's test upon this material; the reaction should be positive.[2] Protid is present. Upon the remainder of the material left in the first test-tube perform the biuret, xanthoproteïc, and Heller's tests. The last test is negative because globin is not heat coagulable.

The hematin portion of the hemoglobin will be discussed later.

Antibodies and Antigens.—When certain substances are introduced into the body, and especially into the body fluids, blood and lymph, they cause the production in the blood of antibodies,[3] which are more or less specific for the substance calling them into being,

[1] For the method of generating CO_2 for laboratory purposes see Appendix.

[2] The test may be obscured somewhat by the yellow color of the hemoglobin. The use of Folin's phenol reagent obviates this difficulty.

[3] Compare the discussion of the Abderhalden Reaction, page 240.

namely, the antigen. As a rule these antigens are protids, but other substances, such as glucosides, lecithins, and others, may also serve as antigens. The antibodies seem to protect the body by rendering it capable of hydrolyzing substances. We know that the simple protids, like protamins and histons, as well as the hydrolytic products of the higher protids, such as the peptons and amino-acids, are incapable of producing antibodies. A certain number of amino-acids must be present in the molecule of a protid before it can serve as antigen. The antibodies may be enzymes. Globin, a histon, does not produce antibodies by itself, but when compounded with another protid,

Fig. 119.—Walter Jones, Professor of Physiological Chemistry, Johns Hopkins University, Baltimore, Md. Investigations of nuclear chemistry (nucleoprotids, purins, etc.).

like caseïn, which has a large number of amino-acids in the molecule, produces an antigen. The physiological reaction known as anaphylaxis[1] or allergy[2] is due to artificial hypersensitization, the term "atopy"[3] referring to natural hypersensitivity.

Nucleoprotids.—These substances are termed "chromatin" by the histologist. They are of exceeding interest from many points of

[1] Greek *ana*, down, and *phylaché*, a guard; that is, breaking down the guard against infection.

[2] Greek *allos*, different, and *ergo*, work, that is, behaving in a different manner.

[3] Greek *a*, privitive, meaning without, and *topos*, place.

view. They give rise to the uric acid of the urine. This is an excellent index of renal efficiency, since it occurs with but slight variation[1] in the normal urine, whereas in nephritis it is retained in the blood, mounting to as high as 20 mgs. per 100 mls. of whole blood. This condition is known, technically, as "retention," which involves also other constituents of the blood, such as urea.

It has been shown by histologists that the nucleoprotids, in the form of "chromosomes" of the cell-nuclei, play an important rôle in heredity and also in the determination of sex (Fig. 120); for there seems to be a quantitative difference between the nucleoprotid of the

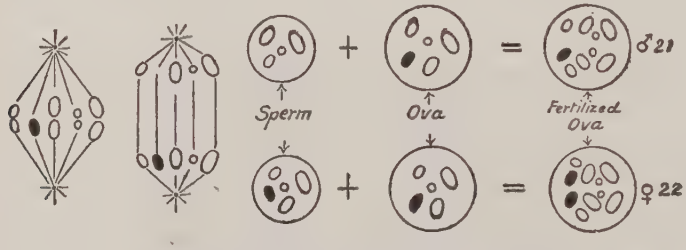


Fig. 120.—Accessory or *x*-chromosome in Anosa. E. B. Wilson, Recent Researches on the Determination and Heredity of Sex. Science, January 8, 1909. Sex determined by the *x*-element. Equally paired chromosome = ♀. Unequally, = ♂. *x*-chromosome black. (From McFarland's Biology, W. B. Saunders Co.)

male and of the female. In certain respects nucleoprotid is one of the most important things in the world, for it involves linking one generation to another. Certain constituents of the nucleoprotids containing nitrogen show a chemical resemblance to "vitamins" which are necessary for growth and for maintenance.[2]

Nucleoprotid is a salt. Its base is a simple protid, composed of strongly alkaline substances, protamins[3] or histons; and the acid is nucleïc acid. If a protamin like salmin be brought into contact with other protids[4] precipitation occurs; and this likewise takes place when protamins and nucleïc acid or histon and nucleïc acid are brought together. When nucleoprotid is slightly hydrolyzed by boiling with acid, protid is cleaved from the molecule, and a substance rich in phosphoric acid is found.

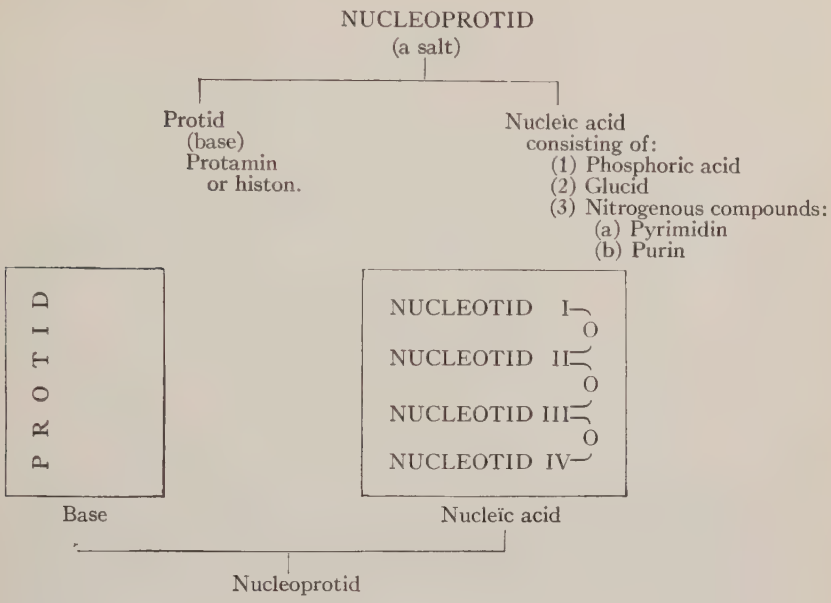
[1] Average normal excretion of uric acid in the urine, 0.7 g. per twenty-four hours. Average normal blood uric acid concentration, 3.6 milligrams per 100 mls. blood.

[2] Page 557.

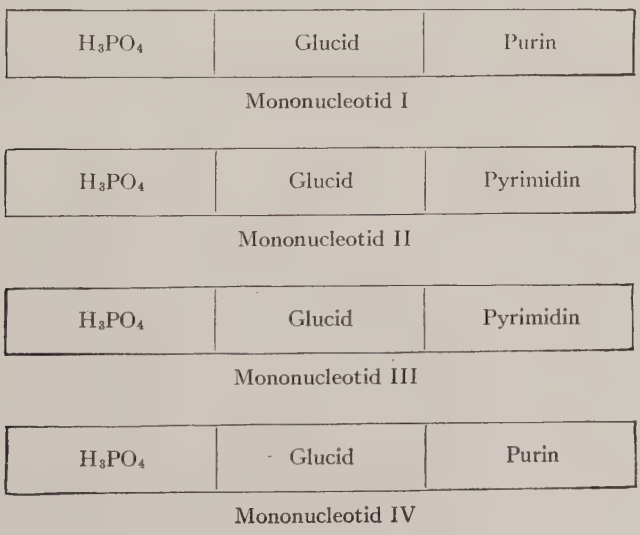
[3] The basicity is so pronounced in protamins that their solution turns red litmus-paper blue.

[4] Page 306.

A graphic representation of the composition of nucleoprotid is given below:

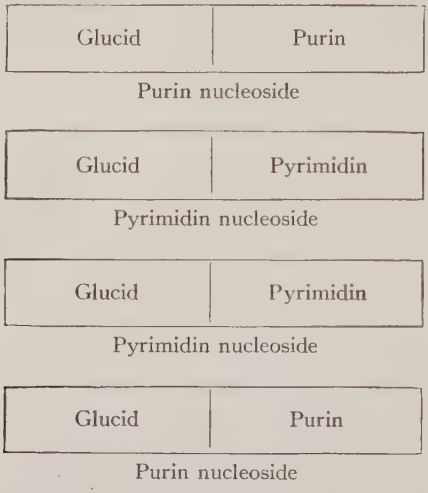


The nucleotids consist of the following components:



The nucleotids may have their phosphoric acid separated from the remainder, leaving nucleosides in which a hexose sugar has as side-

chain or prosthetic group a nitrogenous substance which is either a purin or a pyrimidin, thus:



What substance or substances give rise to nucleïc acid in the body? There is convincing evidence that nucleoprotid is synthesized in the animal body: (1) At every division of the cell there is a restoration of one-half of the chromosomes. Moreover, chemical analysis of eggs, etc., shows that there is a progressive increase in nuclear material as the egg grows.[1] We have stated[2] that the phospholipid, lecithin, is found in rapidly growing and actively metabolizing tissues and if the structure of the two compounds is compared with that of nucleic acid, the similarity in constitution of the two compounds is revealed:

Lecithin contains.

(1) Phosphoric acid, H_3PO_4.
(2) Organic substances:
  (*a*) Glucid derivative, glycerol and fatty acids.
  (*b*) Nitrogenous substance: Cholin (substituted NH_4OH).

Nucleïc acid contains.

(1) Phosphoric acid, H_3PO_4.
(2) Organic substances:
  (*a*) Glucid derivative or the glucid itself: hexose (glucose).
  (*b*) Nitrogenous substance: Purins and pyrimidins.

The chemical composition of nucleïc acid is simple,[3] consisting as it does of four parts known as nucleotids.[4] Hence it is called a tetra-

[1] In the case of the mammal since the developing fetus is supplied with food from the mother, this proof would not be clear, but in eggs, isolated from the parent, like those of the common fowl, fish, etc., the matter is easily decided.

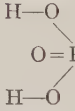
[2] Page 209.

[3] Diagram, page 327.

[4] Greek *nucleo* and *tid*, diminutive, meaning little.

nucleotid,[1] and each nucleotid is a mononucleotid.[2] Each mononucleotid consists of three parts: Phosphoric acid, glucid and nitrogenous portion, purin or pyrimidin, but not the two in the same mononucleotid. On short hydrolysis, the phosphoric acid is split from the mononucleotid and there remains a glucid-nitrogenous portion known as a nucleoside.[3]

The mononucleotids are linked together by means of an ester- or anhydrid bond. The glucid and nitrogenous portions of the nucleosides are joined by substitution, as in the case of glucose and fructose in the molecule of ordinary table-sugar, sucrose.

The Components of Nucleic Acid.—(1) *Phosphoric Acid.*—This is "ortho"-phosphoric acid:  Phosphorus is pentavalent.

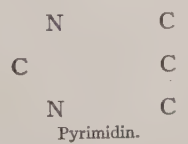
(2) *Glucid.*—It is not known certainly what hexose is present in animal nucleïc acid. In fact, the presence of a hexose is assumed only because its derivatives (levulinic[4] acid, formic acid, etc.) are found in the hydrolysis products of nucleic acid. In the plant, a pentose, ribose, represents the hexose of the animal nucleïc acid.

The glucid is united with phosphoric acid on one hand and with the nitrogenous substance, pyrimidin or purin, on the other. The phosphoric acid association is an ester linkage, as we have stated:

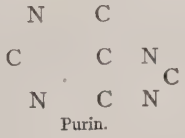
Phosphoric acid ——— O ——— nitrogenous part.

The bond between the hexose and the nitrogenous part is that of a glucoside and is graphically represented as follows: . The link is through the carbon of the primary alcohol of the hexose, CH_2OH, that is the epsilon atom.

(3) *The Nitrogenous Portion.*—Two mononucleotids bear pyrimidins and two carry purins. Since the pyrimidins are simpler than the purins and since the purins are virtually pyrimidin conjugated with a radicle like urea, we shall discuss the pyrimidins first. The two substances are shown structurally:



Pyrimidin.



Purin.

[1] Greek *tetra*, four.

[2] Greek *monos*, one, or single.

[3] Review the glucosides, etc., especially the structure of the diglucid, page 188.

[4] Page 167.

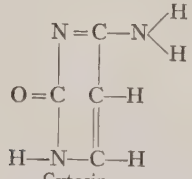
(*a*) *Pyrimidin.*—For reference we may speak of the parts of the pyrimidin as the urea radicle and the carbon chain:

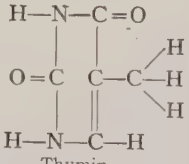
N C
C C
N C
Urea radicle. Carbon chain.

The pyrimidin nucleus is as follows:

$$\begin{array}{l} N^1=C^6- \\ \quad\ |\ \ | \\ -C^2\ \ C^5- \\ \quad\ ||\ \ || \\ N^3-C^4- \end{array}$$

The atoms of carbon and of nitrogen are indicated by numerals. Unsatisfied bonds occur on all of the carbons in the 2, 4, 5, and 6 positions. Different kinds of pyrimidins are formed depending upon whether these bonds are satisfied by an oxygen, an amino- or methyl group. Two and two only occur in the nucleus of the animal cell:[1]


Cytosin
(2-Hydroxy-6-amino-pyrimidin).


Thymin
(2, 6-Hydroxy-5-methyl-pyrimidin).

Properties of the Pyrimidins.—Both of the pyrimidins occurring in the animal cell have been synthesized. They are separated with some difficulty from the molecule of nucleïc acid by acid hydrolysis with increased pressure and after the addition of silver nitrate are obtained as pyrimidin-silver compounds. Cytosin forms plates of crystals not readily soluble in water. It forms single salts with picric acid and double salts with platinic chlorid. It is precipitated by phosphotungstic acid. Treated with nitrous acid, it is deaminized by oxidation and converted into uracil, a plant pyrimidin, fundamental for the other pyrimidins:

$$\underset{\text{Cytosin.}}{C_4H_3N_2O.NH_2} + \underset{\text{Nitrous acid.}}{HNO_2} = \underset{\text{Uracil.}}{C_4H_3N_2O.OH} + N_2 + H_2O.$$

[1] In the plant cell one of the pyrimidins is the same as one that occurs in the animal cell (cytosin), but the second one is different from that which occurs in the animal nucleus (uracil, 2-6-hydroxy-pyrimidin, which replaces thymin of the animal cell).

Thymin is not precipitated by phosphotungstic acid, crystallizes in the form of fine needles or platelets, and does not form salts with acids. The silver salt is soluble in ammonium hydroxid solution.

We have called attention[1] to the fact that the vitamin water-soluble B bears a chemical resemblance to the pyrimidins, and as Williams[2] says: "The chemical reactions and natural occurrence of vitamin B so far as known agree very closely with the pyrimidin bases."

(*b*) *Purin.*—Purins occur not only in the nuclei of cells, but in the tissues, especially in muscle, where hydroxy-purins, the forerunners of uric acid and the end-product of purin metabolism are found in such compounds as hypoxanthin and xanthin. The relationships to uric acid are as follows:

Hypoxanthin,	$C_5H_4N_4O$
Xanthin,	$C_5H_4N_4O_2$
Uric acid,	$C_5H_4N_4O_3$

Purins also occur in methylated condition in plants which are used in the making of beverages, such as caffeïn in coffee and tea and theobromin in chocolate and cocoa. The formulæ are given below in connection with other purins.

Purins are widely used in medicine to stimulate the higher centers, such as the psychic areas of the cerebrum; the medullary centers controlling breathing and vasomotor action; and in larger doses, the reflexes of the lower portions of the central nervous system. They cause quickening of the heart-rate. One of their most characteristic effects is diuretic.[3] Caffeïn occurs in the ordinary cup of coffee to the extent of 0.1 g., the toxic dose of pure caffeïn being about 1 g., although this differs with individuals. Usually 0.5 g. is the maximal therapeutic dose, the ordinary dose being 0.15 g. Caffeïn is taken largely from tea leaves, for commercial purposes. Many races use some alkaloid[4] like caffeïn in beverages. Thus tea is largely used by the Far Eastern peoples, Japanese, Chinese, Siberians, and Russians. Coffee

[1] Page 557.

[2] Williams, R. R., formerly of the U. S. Government service in the Philippines, where he conducted studies in the chemical composition of the vitamins. For a list of publications see Jour. Industr. and Engineering Chemistry, vol. 13, p. 1107, 1921.

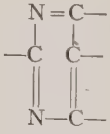
[3] Greek *dia*, through, and Latin *ren*, kidney, that is, accelerating the passage of liquids and dissolved and suspended substances through the kidneys. See page 857 for the relation between diuretics and blood chloride.

[4] The term "alkaloid" referred to such substances as caffeïn, but other, dissimilar substances were included. They are basic (hence "alkali-like;" Greek *alk* + *eidos*, like).

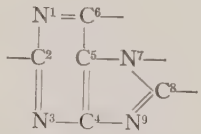
is used universally, but particularly in Europe and Central and South America. The Dutch use large quantities of cocoa containing theobromin, obtained from the cacao-plant, the fat of which is used in the making of cocoa-butter. Some of the theobromin is retained in this butter, which has caused its use as a butter substitute to be prohibited by law. The people of South America, especially of Paraguay, make maté containing caffeïn.

In uric acid we have a most important substance, which will be discussed in detail under the heading Urinary Constituents and Urinalysis.[1]

The Chemistry of the Purins.—Let us start with a plant pyrimidin, uracyl, chosen because of its simplicity of structure. We may convert it by a series of reactions into a purin, like uric acid. To accomplish this, another urea radicle must be introduced by means of nitric or cyanic[2] acid. The structural formula for the purin nucleus is comparable to that for the pyrimidin nucleus:

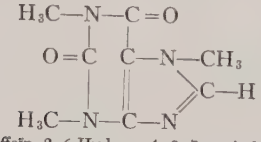


Pyrimidin nucleus.

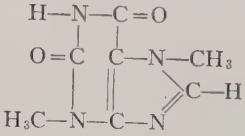


Purin nucleus.

The group consisting of atoms 7, 8, and 9 represents the added urea radicle. By introducing this radicle we have made an iminazol ring[3] consisting of atoms 4, 5, 7, 8, and 9. This configuration occurs in histidin. The substance, purin, which is made by satisfying the bonds on the carbon atoms with hydrogen atoms does not exist in nature, but it has been synthesized by Emil Fischer.[4] All other purins have, in addition to the hydrogen atoms, other atoms or radicles, such as oxygen, amino-, methyl, etc. The two plant purins, caffeïn and theobromin, are methyl-purins, the structure being:



Caffeïn, 2, 6-Hydroxy-1, 3, 7-methyl purin.

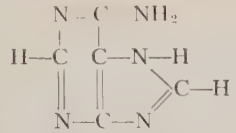


Theobromin, 2, 6-Hydroxy- 3, 7-methyl purin.

Two purins, adenin and guanin, occur in animal cells. These are amino-purins which are made from the purin nucleus by adding

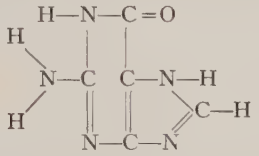
[1] Page 719. [2] Page 708. [3] Page 272. [4] Page 178.

either an amino-radicle to the sixth atom (6), the remaining bonds being satisfied by hydrogen, as in purin:



Adenin, 6-Amino-purin.[1]

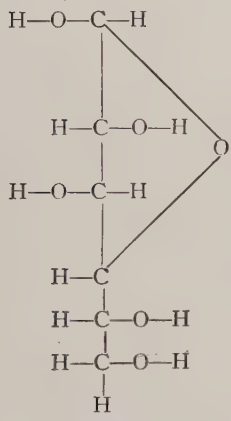
Or, as in the second nuclear purin guanin,[2] 2-amino-6-hydroxy-purin, the amino-radicle occurs on the atom (2) and oxygen replaces the hydrogen of the (6) of purin. Since oxygen is bivalent, it takes one of the bonds from the nitrogen (1), leaving nitrogen (1) with but two satisfied bonds. The third bond must be filled with an atom of hydrogen:



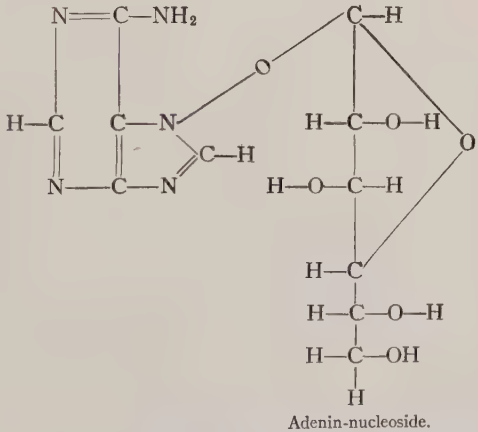
Guanin, 2, amino-6, hydroxy-purin.

The Linkage of the Components into Nucleïc Acid.—Having now considered the several constituents of nucleïc acid, we shall show the manner in which they are united to the molecule (page 335).

Composition of the nucleosides:



Glucose (lactone).



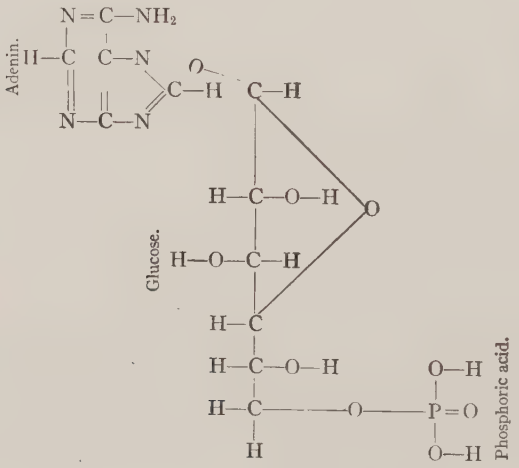
Adenin-nucleoside.

[1] Adenin, so called from the Greek *aden*, gland; it was first isolated from glandular structures.

[2] From the term "guano," the Chilean and Peruvian term for the fossil excrement of sea-birds of the species cormorant, whence guano is obtained. Guano is an extremely important chemical and economic substance. Guanin is the chief form of nitrogenous excretion in the Arthropods (spiders, etc.). For an excellent account of the guano industry, see National Geographic Magazine, vol. 46, p. 279, 1824, article entitled, The Most Valuable Bird in the World, by R. C. Murphy; also article by Coker, June, 1920.

The above formula represents the synthesis of a nucleoside (adenoside) from adenin and glucose. All four nucleosides are synthesized in this manner.

Composition of the Nucleotids.—Phosphoric acid is incorporated into the nucleoside, the primary alcohol, CH_2OH, being attached to the phosphoric acid molecule:



Adenin-nucleotide (adenotid).

In the following graphic representation, we have followed the opinion of Levene (p. 211) whose intensive studies are described in the Journal of Biological Chemistry.[1]

The synthesis of adenotid is accomplished by subtracting water, the hydrogen from the acid (H_3PO_4), and the hydroxyl from the glucose primary alcohol radicle, CH_2OH.

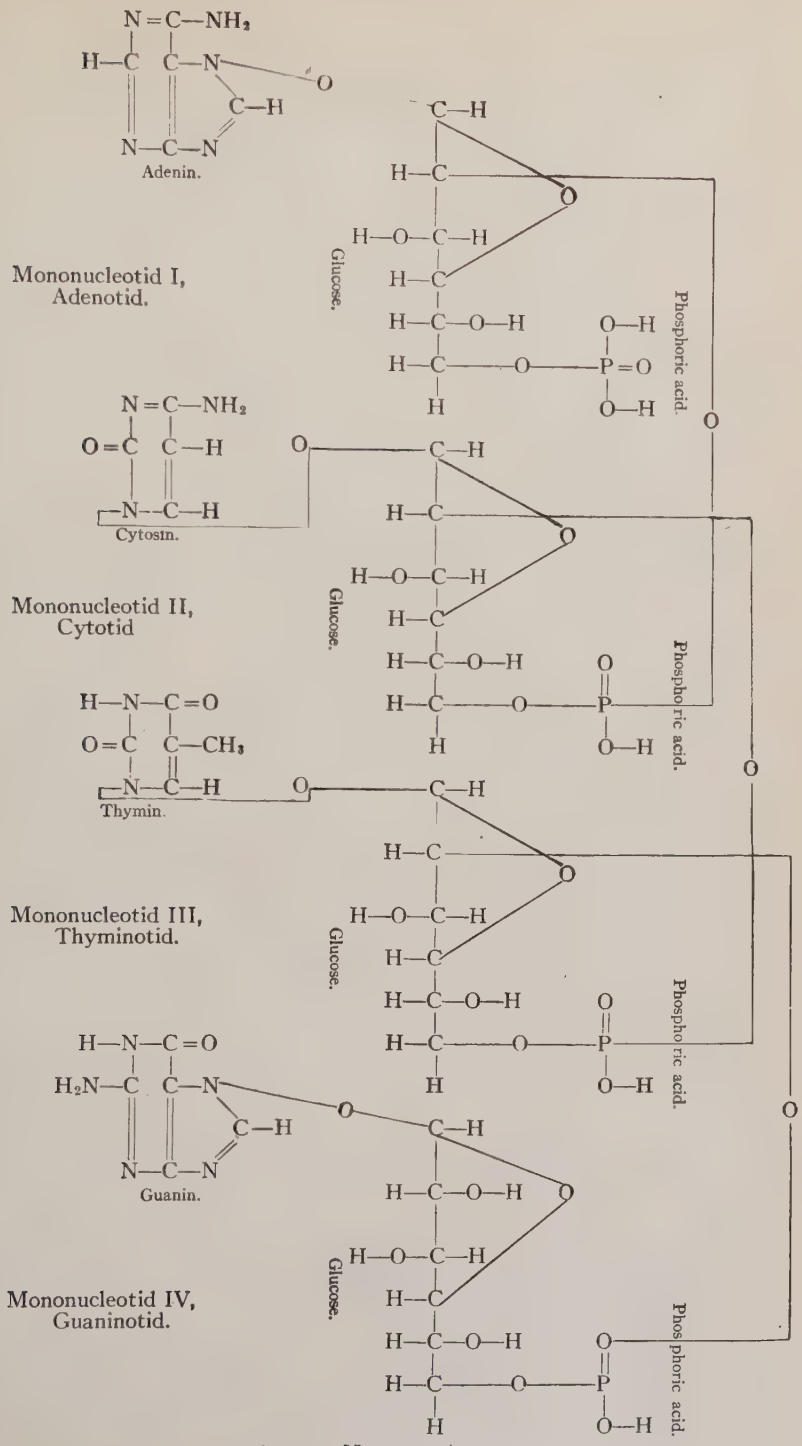
Composition of the Whole Molecule of Nucleïc Acid.—The diagram shown on page 335 is modified from Levene's[2] formula for thymus nucleïc acid.

The Decomposition of Nucleïc Acid.—The purins, occurring in the nuclei of the cells, undergo changes preparatory to their discharge from the body.[3] The process is that of successive deaminizations and

[1] See Levene, P. A. and Simms, H. S., Journ. Biol. Chem., vol. 65, page 519, 1925. Also: Levene, P. A., Bass, L. W. and Simms, H. S., Journ. Biol. Chem., vol. 70, page 229, 1926. References to earlier work are given. The attachment of the pyrimidin is based upon the second paper cited above.

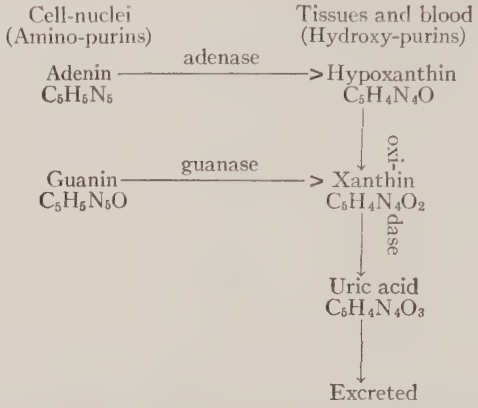
[2] Page 211.

[3] Page 336.



ANIMAL NUCLEIC ACID.

oxidations and is accelerated by specific enzymes. The following illustrates the process:



The oxidase, xanthinase, which oxidizes hypoxanthin to xanthin, and xanthin to uric acid, works in a manner that may be imitated by milk in the presence of methylene-blue. The methylene-blue removes hydrogen (hydrogen acceptor) from water, leaving oxygen to unite with another substance.

The purinases, or purin-deaminizing enzymes, are present in all tissues, but the oxidases affecting purins occur only in the liver. Besides uric acid, methyl purins (caffein, theobromin, theophyllin, etc., of the beverages), derived from the foods are excreted in the urine. They do not arise from the body tissues and escape in some manner the oxidizing action in the liver. Nearly all of the food purins, especially the amino-purins, are oxidized to uric acid. Of the total purin excretion in twenty-four hours (0.72 gm.), only from 0.15 to 0.045 g. represents purins escaping oxidation.

Action of Digestive Juices on Nucleoprotids.—The action may be summarized as follows:

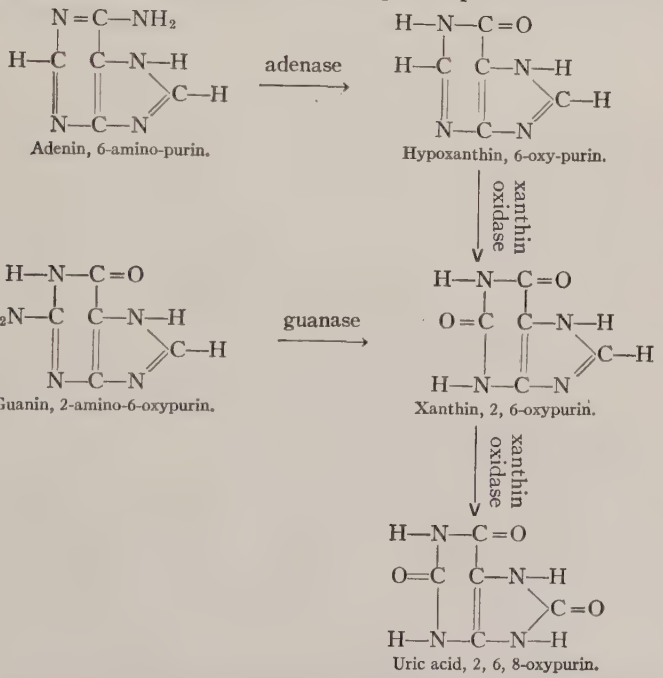
Alimentary Enzymes.—In the stomach the basic protid is hydrolyzed from the molecule of nucleoprotid, leaving nucleïc acid. Pancreatic trypsin causes the digestion of the protid portion of the nucleoprotid more readily than stomach pepsin. Nucleïc acid is affected in the alimentary tract by two enzymes in the intestinal juice as follows:

(1) A nucleinase; this enzyme hydrolyzes the tetra nucleotids, giving rise to four mononucleotids.

(2) A nucelotidase or nucleotidases; this enzyme hydrolyzes the four mononucleotid into four nucleosides and four molecules of phosphoric acid; or, from each mononucleotid may

arise a free purin (adenin or guanin) and a hexose phosphate.[1] In the mucosa of the intestine, all of these enzymes are present and in addition, an enzyme, capable of cleaving pyrimidin from the mononucleotid, but this enzyme is less potent than that which hydrolyzes purins. On page 339 is a graphic summary of purin metabolism.

Tissue Enzymes.—All tissues, without exception, contain enzymes capable of hydrolyzing protids under definite conditions of reaction. Consequently, they are capable of cleaving the protamin or histon from the nucleoprotid, and they also contain nucleotidases and nucleosidases, capable of splitting the nucleotides and then the nucleosides. They are further capable of deaminizing adenin and guanin to make the oxypurins, hypoxanthin and xanthin, but the liver is alone capable of converting hypoxanthin to xanthin and xanthin to uric acid according to modern workers, although recent work[2] indicates that other tissues may participate in this function.



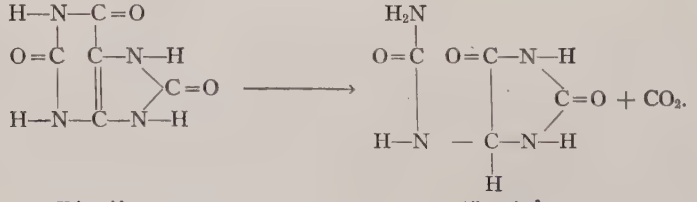
The above set of reactions are extensions of the scheme shown on page 336 for the metabolism of the purins.

Is Uric Acid Destroyed in the Body?—The deaminizing enzymes, adenase and guanase, are quite widely distributed through the vari-

[1] The nucleotidases do not cause the cleavage of pyrimidin from the mononucleotid.

[2] Page 338, note 2.

ous tissues of the body, but the oxidases (grouped as xanthin-oxidase) have a more limited distribution, as we have stated before. The liver in man seems to be the sole seat of purin oxidases. In the lower animals the spleen contains a certain amount of this form of enzyme. The food-purins must pass through the liver in the portal circulation and most of these substances become converted to uric acid in this organ. A small amount of food purin escapes oxidation and becomes eliminated from the body through the urine.[1] All the remaining food and tissue purins become oxidized to uric acid. The question is, What becomes of this uric acid? Does the 0.7 g. eliminated each twenty-four hours represent all of the purin oxidized to uric acid, or is some destroyed in the body? Mann (page 474) by extirpating the liver has been able to show that no uric acid is destroyed unless the liver is present. This is the basis of a test for hepatic efficiency by studying the uric acid, quantitatively. Folin[2] has shown that the blood must destroy a relatively large amount, and some destruction of this acid may occur in the kidneys, which are capable of absorbing much uric acid under normal conditions. When a known amount of the acid is injected into the blood of a dog, 70 per cent. is destroyed in ten minutes. What agent destroys uric acid in the blood and in the kidney is not known. Folin does not believe that it is an enzyme because blood mixed with uric acid and withdrawn from the body does not cause the destruction of the uric acid. The oxidizing agent, apparently, is furnished to the blood by some other tissue and only as required. Under normal conditions all organs except the kidney are practically impermeable to uric acid. This is particularly true of the muscles. The fate of oxidized uric acid is not known. It may become allantoin. The procedure is one of oxidation and hydration, a carbon dioxid molecule being removed:



Uric acid. Allantoin.[3]

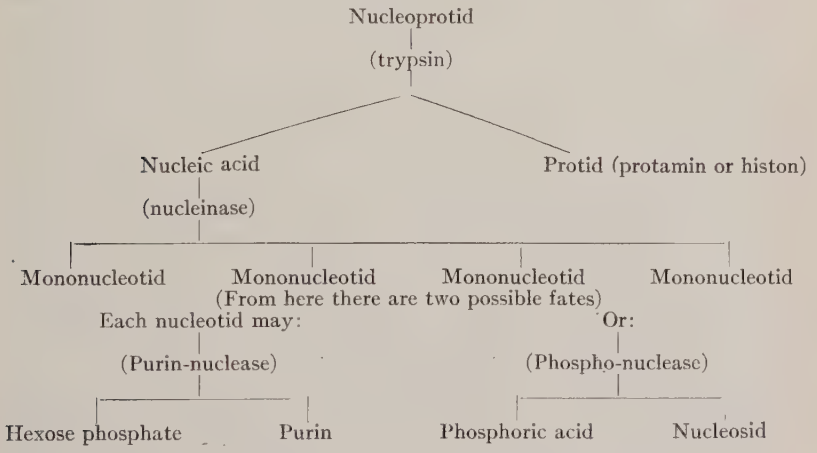
[1] Page 719. Compare this escape of purins with the escape of some of the cystin sulphur which is excreted as the "neutral" sulphur in the urine (page 794).

[2] Folin, O., Berglund, H., and Derick, C., The Uric Acid Problem, Jour. Biol. Chem., vol. 60, p. 361, 1924.

[3] The name allantoin was given by the French chemist Lassaigne to the material he obtained from the allantoïc fluid of the cow (Gr. *allas*, sausage 'oid, like) the allantois being sausage-shaped. Allantoin, however, had been discovered twenty years previously by Vauguelin who obtained it from the amniotic fluid and called it Acide ammotique. Allantoïn is pronounced al-an′ to-een, rhyming with betaïn, etc.

The allantoin nitrogen of the urine is increased in certain pathological states, involving degenerative changes in tissues (diabetes insipidus; muscular changes, as in tetanic conditions). The feeding of purins to a man does not increase the excretion of allantoin, but in the lower animals, like the dog, such an increase does occur. A small amount of uric acid appears in the saliva and in the perspiration, but these amounts are insignificant.

Extranuclear Nucleotides.—It has already been stated that in animals the glucid of nuclear nucleoprotids is a hexose, but two kinds of mononucleotids have been discovered in muscle and other tissues in which the pentose, ribose, is the glucid. These two nucleotids are inosinic acid (H_3PO_4 + ribose + hypoxanthin) and guanylic acid (H_3PO_4 + ribose + guanin). The key to the structure of the nuclear nucleoprotids was given by these nucleotids, representing as they do one of the four mononucleotids of the tetranucleotid, nucleic acid of the nuclei. These nucleotids occurring outside the nucleus are derived from the foods. The fate of the pentose is not known. However, see page 769 concerning pentose excretion.



Practical Studies on Nucleoprotids

Exercise 51.—Place 200 gs. of finely chopped gland material[1] in a liter flask and dilute with 600 mls. of "physiological normal"[2]

[1] For these experiments almost any gland material, such as pancreas, thymus, spleen, lymph-glands, liver, or kidney, may be used, but organs which contain colored substances, like liver, are less satisfactory. Thymus is classic material, which has been used by Levene and others who have studied nucleic acids. It is obtainable in all markets and especially in metropolitan shops. The chemistry of nucleoprotid may be studied by using yeast, and we include as an alternative study a method for analysis of yeast nucleoprotid.

[2] Page 122.

saline solution; then add 5 mls. of toluene to inhibit the action of bacteria. Shake the contents thoroughly and leave in your desk until the next period. If the temperature of the desk is above 20° C., the material should be transferred to an ice-box or other cool place. Then filter first through glass wool[1] and then through paper. Save the filtrate, which is milky in appearance. Dilute the glacial acetic acid reagent on your desk 1:100 and add, drop by drop this diluted acid until the nucleoprotid is entirely precipitated. Too much acid causes a re-solution of the precipitate, which may usually be counteracted by the addition of a drop or two of 10 per cent. NaOH. Place a hardened filter[2] in the Buchner funnel and suck dry. Wash with a small amount of water (25 mls.) to which 5 drops of the 1:100 acetic acid solution have been added; then repeat with 85 per cent. ethanol and with 25 mls. ether (avoiding flames!!), sucking dry each time. Transfer the residue of nucleoprotid to a small beaker or evaporating dish and leave until the following period in a desiccator over sulphuric acid.[3] Weigh the amount of dry nucleoprotid to determine the yield obtained from the 200 gs. of wet gland material. Determine the properties of the nucleoprotid as follows:

(*a*) *Test for Phosphorus.*—Transfer a small amount (1 g.) of the dry nucleoprotid to a small porcelain crucible, place upon a pipestem triangle resting on a tripod; use a micro-burner in place of the usual Bunsen burner. Cover the dry material with as much "fusion mixture"[4] as will lie on a quarter, and by means of a small glass stirring-rod mix the two materials thoroughly. Cover the crucible, but leave an opening of about 1 cm. Heat gently. Fumes will come off and later the mass will turn black. After that the color will disappear, leaving a white mass. Permit this to cool spontaneously with the cover removed, add a small amount of water,[5] and, by means of a stirring-rod, dissolve the mixture. To the solution add a drop of

[1] Glass wool should be handled with the very tips of the fingers if the skin is delicate, for the small glass bristles penetrate the skin and give rise to a mild dermatitis. The proper manner of handling glass wool is to lift a pinch of the long fibers from the container, place it in the palm of one hand and gently roll the mass while the two palms are held about 2 cms. apart, the edges of the palms being in contact. This makes a loose ball, which does not pack tightly in the funnel.

[2] A hardened filter is one which is stiff and tough so that a precipitate can be scraped from it without destroying the surface of the paper. A Buchner funnel is a porcelain funnel with perforated bottom (Fig. 261). The manner of arranging the apparatus for suction filtering is shown in Chapter XVII.

[3] For the best manner of making a desiccator see page 93.

[4] Appendix.

[5] Hot water facilitates the dissolution.

concentrated nitric acid, warm, and then add reagent ammonium molybdate solution,[1] whereupon the yellow precipitate consisting of the double salt (page 360) is obtained. Add 1 ml. of Benedict's hydro-quinon reagent. The production of a blue color shows phosphorus.

(*b*) *Test for Glucid.*—Hydrolyze the nucleoprotid by adding 4 volumes of 10 per cent. H_2SO_4 solution to 1 volume of the dry nucleoprotid in a 200 x 20 mm. Pyrex test-tube. Clamp the tube upon a ring-stand in a vertical position and cover with an inverted crucible cover. Apply the flame of a micro-burner to the bottom of the tube and let the contents boil for an hour or more.[2] The color of the solution will change to light brown, then to dark brown, then black, and finally the shade may become lighter as the "melanin" or condensation products undergo further hydrolysis. If hydrolysis is carried still farther, the glucid may be changed and fail to give the reaction described below. Pour off one-half of the solution into a test-tube and dilute with 1 volume of distilled water. Filter. Use one-half of the solution for the detection of glucid by means of the Benedict qualitative method, reserving the remainder for (*c*).

(*c*) *Test for Protid.*—Apply the following tests:

(1) Biuret.

(2) Folin's phenol test (tyrosin or tryptophan).

(3) Millon's reaction.

(4) Xanthoproteic reaction.

(*d*) *Test for Nitrogenous Constituents; Purins.*—Pour the filtrate left from the hydrolysis in (*b*) into a beaker and add drop by drop concentrated ammonium hydroxid until the neutral point by litmus has been reached. Then make alkaline with a few more drops of NH_4OH. Filter; to the filtrate add 4 volumes of 5 per cent. ammoniacal silver nitrate solution.[3] The precipitate, white or brownish in color, according to the exposure to strong light, is purin silver nitrate. The precipitate is meager at first, but increases with time.

There are several other purins besides the nuclear amino-purins. Hypoxanthin and xanthin will be discussed under the subject "mus-

[1] Appendix.

[2] Care should be taken that the solution does not concentrate; add, from time to time, a few drops (1 ml.) of distilled water to maintain the original volume of the solution.

[3] Appendix. The procedure should be made in subdued light, owing to the reduction of silver.

cle,"[1] and uric acid under the subject "urine."[2] The chemistry of the pyrimidins is not well adapted to the presentation in elementary biochemistry. For methods see Jones.

Phosphoprotids.—Two of the phosphoprotids occur in human biochemistry: Both are concerned with infant food, both secreted by the female; they are also important foods for adults. The first is the ovovitellin of the egg of the fowl and the second is the casein[3] of milk. Phosphoprotids contain phosphorus and it is a common error to confuse them with nucleoprotids. The manner of linkage of phosphorus in the molecule of the phosphoprotid is unknown at present, but it is evidently different from that in the nucleoprotids. No glucid occurs in the phosphoprotid and there is no nitrogenous part other than protid. The phospholipid, lecithin, likewise contains phosphorus, but in the phosphoprotid, there is no fatty acid or glycerol. The percentage of phosphorus in nucleoprotid and in the phosphoprotid is similar:

	Gs. per cent.
Phosphorus estimated as P_2O_5 from nucleoprotid	1.05
Phosphorus estimated as P_2O_5 from phosphoprotid	0.85

The amino-acid content of these protids is quite complete, as would be surmised from the rôle they play in the nutrition of the growing animal. They contain amino-acids qualitatively and quantitatively necessary for the development of offspring.

Exercise 52.—Prove that phosphorus occurs in the molecule of the sample of purified caseïn given you[4] and also that it occurs in the lipid-free ovovitellin sample submitted. Likewise demonstrate the protid nature of these compounds.

THE HYDROLYSIS PRODUCTS OF THE PROTIDS—PROTIDTEMNS

Frequently protidtemns are called "derived protids." We have already discussed these products briefly in connection with the hydrolysis of meat,[5] but will go into more detail here. The following classification serves to bring out their chief characteristics:

[1] Page 370.
[2] Page 719.
[3] Page 411.
[4] For the method see page 360. Pure caseïn can be obtained from the Harris Laboratories, Tuckahoe, N. Y.
[5] Page 235.

I. Heat-coagulable Products.—First hydrolysis products produced by catalyzers like water, dilute acids, or alkalis and enzymes. Changes typically colloidal in nature but of hydrolytic origin.

A. *Alloprotids.*[1]—Insoluble in excess of the reagent. Sulphur is not removed. Examples: Alledestin; allomyosin; alloglobulin.

B. *Metaprotids.*—Soluble in excess of the reagent, but insoluble at the iso-electric point. Sulphur is removed, especially in strongly alkaline medium. Examples: Acid albuminate; alkali albuminate.

II. Products Not Heat Coagulable.—Later products of hydrolysis.

A. *Albumoses.*—Biuret typical, violet predominating. Precipitated by ammonium sulphate solution. Examples: Albumose; globulose.

1. Primary albumoses. Precipitated by half-saturated $(NH_4)_2SO_4$ solution.

(*a*) Heteralbumoses. Precipitated by adding 1 volume of 95 per cent. ethanol; precipitated by 32 per cent. ethanol. Soluble in water.

(*b*) Protalbumoses. Precipitated by alcohol more than 80 per cent. concentration. Soluble in water.

2. Precipitated by complete saturation of $(NH_4)_2SO_4$. No precipitate is formed with concentrated nitric acid added drop by drop, nor by potassium ferrocyanid in dilute acid solution. Secondary albumoses.

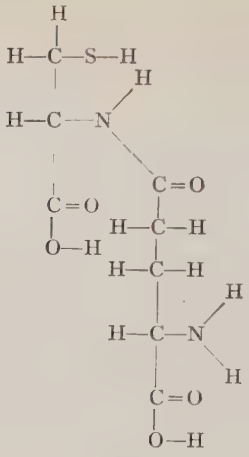
B. *Peptones.*—Not precipitated by ammonium sulphate in any concentration. Biuret atypical, pink predominating.

Final Products of Protid Hydrolysis.—These comprise (1) the *polypeptids*, or lower aggregates of amino-acids, peptons and albumoses being higher or more complex linkages; and (2) *amino-acids*. Theoretically, all polypeptids consisting of more than two amino-acids (tri-peptids, tetra-peptids, etc.) are protids, since they respond to the biuret test, which is given by substances having two or more biuret groups. However, we have already defined protids as colloidal polypeptids.[2] Of this we shall speak later. One of the most important polypeptids is glutathion[3] which is a condensation product of two amino-acids, glutamic acid and cystein. The formula is:

[1] Frequently called proteans, but the suffix *-an* has long been used to designate the polyglucids (page 154). The author proposes to use the prefix "*allo*" for the first products of protid hydrolysis, from the Greek *allos*, others.

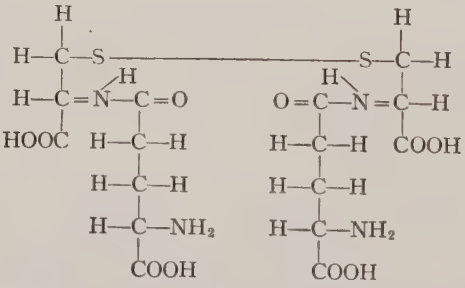
[2] Page 302.

[3] Page 257.



Glutathion.[1]

On being oxidized, glutathion, which chemically is the di-peptid glutamylcysteïn, becomes condensed as two molecules to form diglutamyl-cysteïn, or glutamyl-cystin:



The Physical Condition of the Protid Hydrolysis Products.—The protidtemns including and above the albumoses are colloidal and do not diffuse through a membrane; those below and including the peptons are crystalloidal and pass through membranes. Absorption of the protid products begins with the peptons and includes polypeptids and amino-acids. Typical absorption of protid food takes place in the form of amino-acids, but the lower peptids are absorbed to some extent. Albumoses are toxic and retard coagulation of the blood. In small amounts they increase the blood-pressure and in larger quantities decrease it. It is improbable from such considerations that albumoses are absorbed to any extent. When injected into an experimental animal, albumoses cause local reactions, fever and lesions of the organs, such as cirrhosis of the liver, etc. Albumoses do not

[1] Formula of Quastel, J. H., Stewart, C. P., and Tunnicliffe, H. E., 1923, Biochem. Jour., vol. 17, p. 586, 1923. See also same Journal, vol. 19, pp. 194, 199. Also p. 207.

act as antigens. Pathologically, they occur in the blood and in the urine, producing "albumosuria," as in the absorptive stages of pneumonia, following the crisis, in which extensive autolysis[1] is believed to occur.

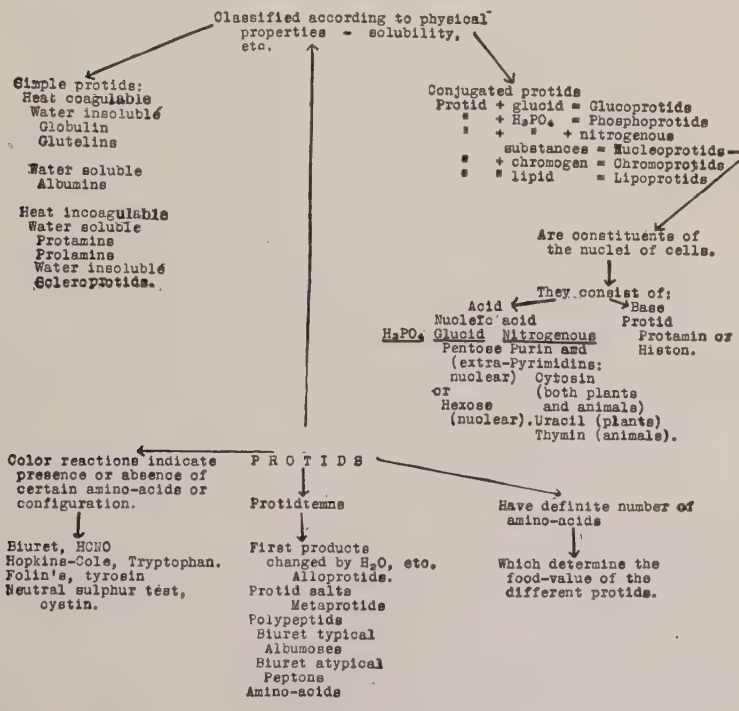
SUMMARY FOR PROTIDS[2]

1. The classification of protids presented here is based chiefly on physicochemical factors.

2. There are three principal groups: Simple, conjugated, and derived protids. The first comprises single protids, or those which, on hydrolysis, do not produce protid and an additional product other than protid. The second group consists of protids capable of being hydrolyzed to protid and to an additional "prosthetic" group. The derived protids are modified protids on their way to complete hydrolysis (amino-acids).

3. Special physiological or pharmacological properties are found in the different hydrolysis products of protids, toxicity being inversely proportional to the degree of hydrolysis; that is, the higher aggregates of amino-acids are more toxic than the lower ones.

GRAPHIC SUMMARY—PART TWO



[1] Page 461.

[2] Page 302.

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CHAPTER VII

THE SPECIAL CHEMISTRY OF THE TISSUES

"So poor is nature with all her craft that from the beginning to the end of the universe she has but one stuff to serve up all her dream-like variety."—*Emerson*, "Nature."

Specialization.—The organism is a great composite of individual units, the cells, and there is a specialization of chemical constitution comparable to the morphological specialization. Probably it would be more exact to say that comparable to the fundamental chemical specialization there is a morphological one, for it is the chemical distribution which defines structural characteristics. Each chemical element and compound exerts characteristic effects, and these effects are expressed in a vast variety of the structure and function of living things. Yet, as Emerson has said, there is a monotony in the composition of the organism, comparatively few chemical "stuffs" composing the great multiplicity of form and function.

The Chemistry of the Tissues and the Germ-layers.—The embryo is composed, fundamentally, of three germ-layers: Ectoderm, mesoderm, and endoderm. The organs arising from these layers have certain characteristic chemical features and it is possible to trace both chemical and morphological relationships. Thus, the ectoderm consists of two typical parts: Secretory cells and protective structures. In man an extreme specialization has occurred whereby the typical secretory cells have become limited in distribution and the protective portions modified. The mucous glands which occur in the skin of the lower forms of life have been limited in man principally to regions where they are protected, as in the invaginated buccal and proctodeal regions, buccal cavity, rectum, vagina, etc. The protective portions mentioned above accompany the invaginations of the glandular portion; characteristic of this protective layer is keratin, and in the central nervous system, which is derived by invagination from the ectoderm, we find neuro-keratin. Ovokeratin occurs in the mucous secretions of the oviduct of the fowl; this form of keratin is passed into the hen's egg before the shell is deposited and we encounter the keratin in the white of egg. Recently, it has been demonstrated that keratin contains relatively large amounts of cystin which is one of

two amino-acids (phenylalanin being the other) that are opaque to ultraviolet light; keratin thus protects both mechanically and chemically.

Again, the structures derived from the mesoderm are typically supporting in function; examples are connective tissues, cartilage, and bone. The secretory portions of the salivary glands are derived from the mesoderm. It is of interest that the secretion of these glands contains chemical compounds which are also characteristic of such structures as cartilage. These compounds are the glucoprotid mucin[1] and chondroitin-sulphuric acid.[2]

STRUCTURES DERIVED FROM THE ECTODERM

The Integumentary System.—The chief function of this system is protective. We should, therefore, expect to find chemical compounds refractory to water and to harmful reagents likely to come into contact with the skin. The skin has two portions: (1) Epidermis, derived from the ectoderm and (2) dermis,[3] from the mesoderm. The function of the dermis is to subserve the epidermis, which is concerned with the making of protective substances composing the cuticle, hair, nails, etc. There is a sex-difference in the content of the characteristic inorganic substance, sulphur, the female having less than the male. Calcium metabolism is on a high plane in the epidermis, and as age advances the calcium content of keratin increases. This is probably a part of the excretory function of the hair, for arsenic, chlorids, and other inorganic substances and organic materials, like cholesterol, are also found in the hair. Formerly this may have been purely for excretion, but later it gave the body the hair.[4] What has been said of the hair applies largely to the nails, since their embryonic origin is similar.[5]

The Pigments of Hair.—Two distinct pigments occur in the epidermal structures of man, namely, black and brown. The variety of color found in the hair of different individuals and races depends upon the distribution of these pigments. Red hair is a diluted black. Gray hair is devoid of pigment and the whiteness is augmented by the inorganic content, principally by the calcium phosphate and carbonate, which are found in larger quantities as age progresses.

[1] Page 320. [2] Page 354. [3] Also called corium.

[4] The erectile function of hair is performed by means of small muscles attached to the base of the hair, but without the stiffening of the ramus of the hair these muscles cannot perform their duty.

[5] It is believed by many biologists that hair, nail, and horn are practically the same structure, the hair in time becoming matted and forming nail and horn.

Through the work of Bertrand,[1] von Fürth, Gortner, and others it has been shown that hair pigments are due to oxidation of a chromogen, or substance capable of being converted into a color-bearing portion. The aromatic amino-acids like tyrosin form the basis of such conversions. Tyrosin is converted on oxidation into melanin substances. Of these there are two classes, one soluble and the other insoluble, in dilute acids. The acid-soluble substances resemble acid albuminates and are dissolved or included in the keratin of the hair. The insoluble melanins are of a different kind and probably have a different origin, for they are not protids.[2] The pigments of the skin and of the iris are chemically the same as the hair pigments. In certain pathological states there is a deposition of pigment, as in the melanotic sarcomas and in retinitis pigmentosa.[3] In others the power of making melanin is lost, as in albinism, healed tissue after severe lesions, bacterial and mould infections, and in syphilis. Melanin becomes clumped into small areas as in freckles during exposure to actinic rays—either those of the sun or of the mercury-vapor quartz lamp. There is probably some nuclear derangement in such cases and the accumulation of pigment is a protective measure. The function of the epidermis to secrete pigment persists when the ectoderm becomes incorporated into interior structures like the nervous system. Pigmentation also occurs in the retina of the eye and in the "substantia nigra" of the brain, both of which are derived from the ectoderm. The Nissl-bodies of nerve-cells are of the nature of pigment, but their composition is unknown. In Addison's disease[4] the adrenal glands are affected, and substances which would otherwise contribute to the formation of epinephrin pass into the blood and are excreted into the skin where they take on the bronze color characteristic of the disease. Care should be exercised, however, in assigning all pigmentation to these melanins, for the pigmentation in malaria, in ochronosis,[5] and in other conditions is of entirely different origin, being obtained from blood-pigment.

[1] Bertrand, Gabriel, contemporary Parisian biochemist; Otto von Fürth, contemporary Austrian biochemist; Ross A. Gortner, see page 266.

[2] There is no contradiction in this statement from that made above where melanins are said to be derived from tyrosin; phenols other than tyrosin occur in the human body.

[3] A disease due in many cases to consanguineous marriages; the retinal cells become pigmented.

[4] Addison, Thomas, English physician of the 19th century (page 658 and page 657, note 4.

[5] Greek *ochros*, yellow. The pigmentation affects the scleroprotids and also pigments become excreted into the urine.

It is of interest that pigmentation is inherited. Thus the brown of the Mediterranean races is dominant[1] over the recessive[1] light hair and blue eyes of the northern races.

The Dermis.—The glands which penetrate the dermis are of entodermal origin. They secrete the oily substances occurring on the surface of the skin, known as sebum.[2] This consists of a mixture of various organic substances, such as a phosphoprotid resembling vitellin; a heat-coagulable protid of the character of an albumin and lipids other than neutral fat. Cholesterol and lipids of unsaturated nature occur. In pathological states sebaceous cysts become filled with esters of fatty acids and higher alcohols, like cetyl alcohol. The skin of the newborn child is lubricated with a fatty substance called vernix caseosa[3] consisting largely of cholesterol and its esters. Lanolin[4] used in pharmacy for making ointments, consists of cholesterol-esters derived from the skin of the lamb. The hair and, to a greater extent, the nails take up cholesterol from the skin probably mechanically. In the ear, a brownish wax known as cerumen[5] is chemically similar to sebum, although it is mixed with the secretions of the sudoriferous glands.[6] Like the secretions of the sebaceous glands, cerumen contains lipids and derivatives, esters, and soaps. The characteristic pigment is responsible for the peculiar bitter taste. Finally, the vaginal and penial secretions are simply products of glands similar to those of the skin. Chemically, they do not depart from the secretion of the skin glands, although there are admixtures of other substances derived from the accessory glands of the genital and urinary system.

The Perspiration.—The skin in the past has been considered chiefly an organ of excretion. Its excretory function is artificially augmented in certain diseases in which the work of the kidneys must be lightened, as in uremia and excessive retention.[7] In such cases

[1] The terms "dominant" and "recessive" are used by geneticists to designate the condition in which a structure or color becomes active, or evident (dominant), or suppressed (recessive) when two animals having different characteristics are interbred. Thus, in the case of pigmentation, if a true Saxon marry a Spanish Castilian of pure descent, most of the children will be largely of dark complexion in the theoretical ratio of 3:1, the Saxon color is transmitted to one child in four, statistically. The dark of the Mediterranean races is dominant over the fair complexion of the northern race in such matings.

[2] Latin *sebum*, animal fat or suet.

[3] Latin *vernix*, varnish and casein; that is, a cheesy varnish.

[4] Page 215. [5] Latin, meaning ear-wax. [6] Latin *sudor*, sweat, and *fero*, to carry.

[7] Retention, see page 229. Uremia, page 682, note 6. For the more recent conception of the function of the skin see Jour. Amer. Med. Assoc., vol. 82, p. 968, 1924.

sweating is produced by means of sudorific drugs like pilocarpin,[1] or by hot baths. In either case the peripheral regions are stimulated and increased metabolism of the skin results. The rôle of the perspiration in water excretion may be seen from the following table:[2]

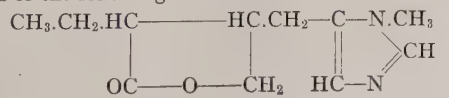
WATER BALANCE (TWENTY-FOUR HOURS)

Intake:	
Drinking water	600 gs.
Beverages like tea, coffee, milk	580
In solid food	720
In 100 gs. of protid	50
In 100 gs. fat	118
In 224 gs. glucid	135
Total intake	2203 gs. water
Outgo:	
Urine	800 gs.
Skin	680[3]
Respiratory tract	600
Feces	200
Total loss	2280 gs.

The perspiration consists of 98 per cent. water and 2 per cent. solids, sodium chlorid predominating (1 per cent.). Organic substances like cholesterol, uric acid, urea and other ones characteristic of the urine have been found. As the amount of perspiration increases, the inorganic radicles, chlorids, phosphates, and sulphates, increase, while the organic compounds decrease. Chlorids occur in perspiration to the extent of 3.2–6.5 mgs. per ml. Total nitrogen averages 0.388 to 1.65 mg. per ml. These two quantities vary directly. Urea (nitrogen) occurs to the extent of 0.05–1.12 mg. per ml. Ammonia (nitrogen) is 0.04 to 0.21 mg. per ml.[4]

The acidity of the perspiration is due to the fatty acids which are hydrolyzed and oxidized from the lipids during the process of excretion. The accumulation of urea and other nitrogenous substances

[1] Pilocarpin, derived from the plant called by the botanist Pilocarpus and by the natives of Central America, jaborandi. Chemically, pilocarpin is an iminazol-ring compound of the following formula:



[2] From Rowntree: The Water Balance of the Body, Physiol. Reviews, vol. 2, p. 122, 1922.

[3] Figure of Gephart and DuBois, Archives Internal Medicine, vol. 17, p. 902, 1916.

[4] Talbert, G. A., Harris, E. E., Finkle, J. R. and Silvers, S. H., Amer. Journ. Physiol., 1926, Proc., p. 185.

when the skin is not bathed and the ammonia and ammonium compounds resulting from deaminization by bacteria of the urea, etc., may render the perspiration alkaline.[1] However, at times an actual alkaline perspiration, fresh from the glands, occurs. Gases like carbon dioxid normally pass from the skin; the average amount given off in this way is 1.5 per cent. of that exhaled through the lungs.

One of the chief functions of the perspiration is that of cooling the body by evaporation; this will be discussed later.

The Mammary Glands.—These are functionally sex-linked[2] integumentary organs; but structurally they occur in both sexes, although functioning has rarely been observed in the male. At puberty the male glands cease to grow, the female persisting.[3] The gland arises in the embryo from the milk-ridge, an epidermal thickening, which later contains both sudoriferous and sebaceous glands; consequently, the secretion partakes of the chemical characters of the two types of secretion. The chemistry of the glands themselves resembles that of the integument as a whole, although the epidermal portions are of less importance. Being a gland, nucleoprotid occurs in conspicuous amounts. There are two types of nucleoprotid: (1) Mononucleotids which occur outside the nuclei of the cells[4] and (2) the true nuclear nucleoprotid,[5] the tetranucleotids, described previously. Besides these sources of phosphorus, milk contains the phosphoprotid caseïn which adds to the phosphorus content of this fluid.[6] It is not definitely known how caseïn is made in the mammary gland, but it is evident that the elements necessary for its synthesis are present in the gland.

The chemistry of milk is discussed on page 409.

The Teeth.—An inspection of the mouth of a shark is instructive as to the origin of the teeth and their relation to the integumentary system. The protective "shagreen" of the skin of the shark passes with imperceptible changes to the oral cavity, where spines borne on the plates of this system become enlarged to form the teeth. Hence we include the teeth in the integumentary system for the purpose

[1] This may be a factor in the readiness with which unclean skin becomes infected, for an alkaline reaction is favorable to bacteria, some moulds, and wild yeasts.

[2] That is, confined to one sex.

[3] Starling, (E. H., Foulerton Research Professor of the Royal Society, London) has demonstrated the direct relation between sex organs and these glands.

[4] These are the "beta-nucleotids" of the English student; they are free mononucleotids (see page 339).

[5] "Alpha-nucleoprotids;" page 334.

[6] Compare muscle phosphorus, 0.04 g. per cent. dried muscle; mammary gland phosphorus, 0.27 g. per cent.

of chemical discussion. Embryologically, both ectoderm and mesoderm are concerned in the formation of the teeth. From the former arises the enamel, the densest material of the body; from the latter the dentine and the pulp.

Chemically, the tooth resembles bone.[1] The cement is true bone. Dentine is similar to bone, but contains less water. Both contain a gelatin-producing substance, collagen, discussed below. Enamel in the older tooth contains no demonstrable water, but in the young tooth from 1 to 2 per cent. of water occurs. The enamel is covered with a thin cuticle, called Nasmyth's membrane,[2] corresponding to the cuticle of the skin. Fluorin occurs in the enamel in small amounts (0.1 per cent.)[3] The principal difference between teeth and bone is the water content. Magnesium seems to occur in larger quantities in teeth than in bone. Under the heading of "deficiency diseases" will be discussed the modification of the teeth with different kinds of foods (page 576).

THE MESODERMAL STRUCTURES

The mesodermal structures comprise such substances as bone, cartilage, connective tissues, etc., and are largely concerned with supporting the organs and tissues of the body.

Cartilage.—In cartilage and allied substances the intercellular material is of especial importance. This is a secretion of the cells in which they are embedded. The characteristic chemical substance of this intercellular material is collagen,[4] which affords the gelatin of commerce. The collagen is laid down in fibers, or fibrils, the interstitial substance between them containing, principally, a mucoprotid, an ester of a protid of unknown nature and sulphuric acid, in the form of the so-called chondroitin sulphuric acid.[5] The composition of this substance is analogous to that of other conjugated protids,[6] *e. g.* nucleoprotids, in which protid is linked with a prosthetic group. The

[1] Just as bone has an organic matrix, teeth have likewise. Gies and collaborators (page 24) have been able to dissolve the inorganic substances, leaving the organic substratum.

[2] Scotch physician, died 1847.

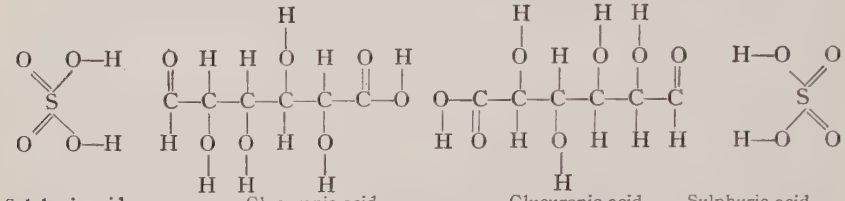
[3] McCollum (page 547) has shown that increased amount of fluorin in the diet of experimental animals tends to cause soft teeth, especially incisors. The use of fluorin (in the form of sodium fluorid and the like) as preservative for foods is perhaps hygienically improper.

[4] Page 307. Collagen is shipped from Russia and other countries in the form of hoofs, etc., and made into gelatin in the United States. This gelatin is now of the highest purity, and when placed on the market is of very low bacterial content. Much commercial gelatin is obtained from skins.

[5] Greek *chondros*, gristle, and *eidos*, like.

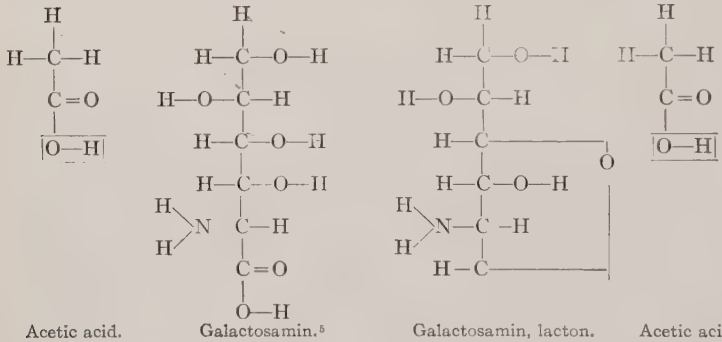
[6] Compare page 303.

exact relationship is unknown, but through the investigations of Levene[1] a tentative structural formula has been derived. This indicates: (1) That a conjugation of two molecules of glucuronic acid with a molecule each of the amino-sugar called *galactosamin*[2] occurs; (2) that the amino-radicles of the glucosamin become acetylated by the addition to each one of a molecule of acetic acid, and (3) that an ester is formed with the last[3] carbon atom, the primary alcohol radicle, CH_2OH, with sulphuric acid. The Levene-LaForge[4] formula is:



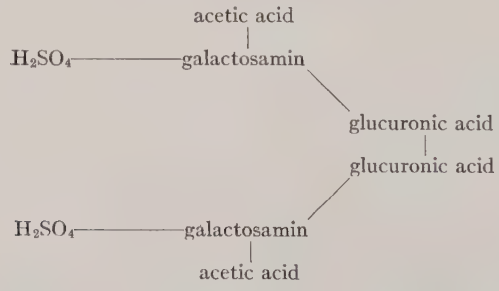
Sulphuric acid. Glucuronic acid. Glucuronic acid. Sulphuric acid.

Components of chrondroitin sulphuric acid.



Acetic acid. Galactosamin.[5] Galactosamin, lacton. Acetic acid.

The molecule may be shown, diagrammatically, as follows:



[1] Page 211.

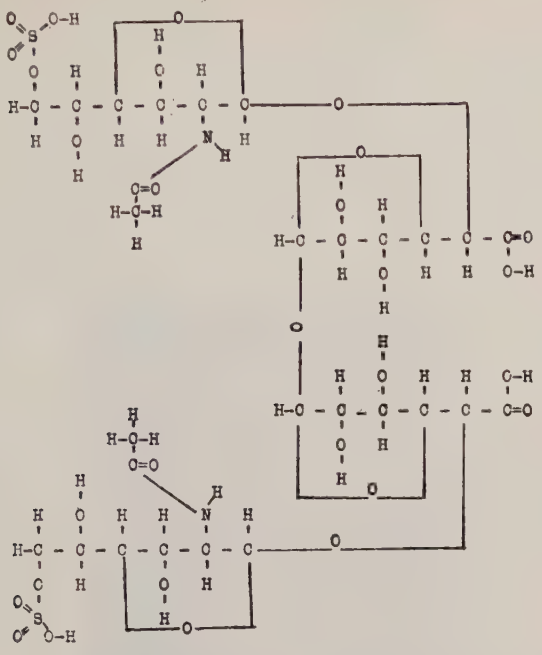
[2] Or *talosamin;* compare talose and galactose, page 179. Identification of these sugars is difficult.

[3] The epsilon carbon atom, the counting being made from the aldehyde radicle.

[4] LaForge, B., 1882–. With the Bureau of Chemistry of the U. S. Department of Agriculture.

[5] The formula is inverted from that used in previous pages, owing to easier rendering of the completed formula for chondroitin sulphuric acid.

The completed formula is as follows:



The mucoprotid is slightly acid in properties. It is insoluble in water, but soluble in dilute alkali solution, such as lime-water. From such a solution the mucoid is precipitated by the addition of a few drops of 10 per cent. hydrochloric acid or by the following reagents in aqueous solutions: Lead acetate; "basic" lead acetate;[1] potassium aluminium sulphate, and ferric chlorid. It is not precipitated with tannic acid or potassium ferrocyanid. Chondroitin sulphuric acid is likewise acid in reaction in aqueous solution. Prepared in the laboratory, it assumes a high viscosity as it becomes concentrated. Unlike tendomucoid, it is not precipitated from solution by dilute "strong" acids nor by concentrated solutions of weak acids (organic acids). It resembles tendomucoid in not being precipitated by the substances mentioned above (lead acetate, etc.). As cartilage becomes older, a substance known as "albumoid" of protid nature appears. This may be obtained by heat coagulation of a cartilage solution in dilute alkali. The collagen is soluble, whereas the albumoid is insoluble and may be filtered off after the alkli has been partially neutralized.

The water content of "green" bone, that is, extracted with ether and then dried in an oven after removal of the marrow, is about 8.5

[1] Page 228.

per cent. for subjects from twenty to sixty years and 8.9 per cent. for those whose age is from sixty-one to ninety-one.[1] Here, as in cartilage, the intercellular matrix is characteristic. It consists of inorganic substances, known as "bone earths" infiltrated into an organic, cartilage-like material called "ossein." The latter, like cartilage proper, contains mucoid and albumoid, with properties similar in every respect to those of cartilage itself. The proportion of ossein, according to Radasch, averages as follows: From twenty to sixty years, 40.7 per cent.; from sixty-one to ninety years, 42.32 per cent. of green

Fig. 121.—F. S. Hammett, member of Research Staff Lankenau Hospital Research Institute. (Photograph by Science Service.)

bone. For study one may remove the bone-earths from bone by soaking the latter in 5 per cent. hydrochloric acid solution. The bone becomes pliable and the inorganic materials may be recovered from the solvent. There seems to be little doubt that ossein, mucoid, and albumoid are identical with the corresponding substances of cartilage; in fact, bone may be thought of as cartilage matrix with a deposition of inorganic substances replacing portions of the matrix.[2]

The following statements summarize the findings of Hammett in a study of bone metabolism in experimental animals: The degree of ossification of bone is greater in the female than in the male, the calcium content being greater in the female than in the male. The

[1] Radasch, H. E. (page 437), Anatomical Record, vol. 21, p. 153, 1921.

[2] The osteoblasts are especial cells the function of which seems to be (1) to remove cartilage and (2) to deposit by active metabolism inorganic substances. Similar cells are found in the deposition of calcareous and silicious skeletons in the lower animals. (See Fig. 52.)

water content of bone decreases proportionately with age, due to ossification. An increase in Ca and decrease in Mg. and P occur up to the end of puberty.

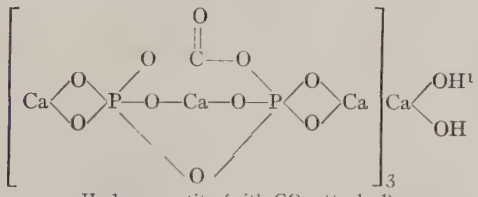
ANALYSIS OF BONE ASH
(Gabriel)[1]

CaO	51.31
MgO	.77
K_2O	.32
Na_2O	1.04
H_2O (crys.)	2.46
H_2O (comp.)	1.32
P_2O_5	36.65
CO_2	5.86
Cl	.01
Total	99.75

[1] Gabriel, S. Chem. Untersuch. ü. d. Mineralstoffe des Knochen und Zähne. Zeitschr. f. physiol. Chem., vol. 18, p. 257, 1894. The humerus was used.

The "bone-earths" are given in the following summary of bone chemistry:

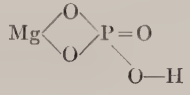
(1) Hydroxyapatite: 88 gs. per 100 gs. bone-ash.



Hydroxyapatite (with CO_2 attached).

(2) Carbon dioxid, as carbonate attached to the phosphate (shown above).

(3) Magnesium phosphate, 2 per cent.



(4) Calcium fluorid, 0.07 per cent.

F—Ca—F

(5) Calcium chlorid, 0.4 per cent.

(6) Iron, Fe^{+++}, 0.1 per cent.

[1] From the work of Bassett, H. Jr., (Univ. College, Reading). Jour. Chem. Soc., vol. 111, page 620, 1917. Bassett showed that such a compound alone is stable in such media as blood, etc. Basset concludes that bone phosphate is a mixture of hydroxyapatite and calcium carbonate with small amounts of adsorbed bicarbonates of sodium, potassium and magnesium. The small amount of chlorin is absorbed as NaCl or as chlorapatite.

The manner in which these various inorganic constituents are combined in bone whether chemically or physically is not known, but it is probable that the carbonate and calcium phosphate are united and perhaps the magnesium compound is linked with them to form a large molecule. The phosphate is probably linked with hexose (fructose) as a hexosephosphate which is hydrolyzed by a special enzyme abundant in ossifying cartilage, but insignificantly in non-ossifying tissue.[1] It is interesting in this connection that after phosphorus poisoning a patient craves sugar.

Iron and coloring-matters are probably of exotic nature; that is, they do not belong to bone, but are brought in blood, or incorporated with fat, which constitutes about 2 per cent. of bone, occurring principally in the marrow. The amount of inorganic substance deposited varies according to age, sex, and other conditions. Thus one of the injuries suffered by a child delivered with instruments is fracture of the cervical vertebræ, owing to the fact that these bones are more highly ossified than those in other parts of the body at birth. As growth continues, ossification of the long bones rapidly takes place, there being a general increase in solids and a decrease in water. In certain diseases involving bones, such as rickets,[2] craniotabes, etc., there is a loss of inorganic portions of the bone. There are two causes for rickets in children:[3] (1) Disproportion of calcium and phosphorus in the diet; (2) lack of an agent causing utilization of these inorganic substances, chiefly calcium. There are three means of administering curative effects derived from (2); these are (1) direct sunlight; (2) artificial light, and (3) foods that seem to carry or to be especially affected by such light. The light must bear rays of fairly definite wave length, the optimal effect being obtained by light of 2900 Å.[4]

Lesions of certain organs cause modifications of the inorganic content of bone and of other parts of the body, such as blood. Thus an increase in the deposit of bone-earths occurs in the disease known as acromegaly,[5] when the gland in the base of the skull known as the

[1] Page 176. The optimum *p*H for this bone phosphoric esterase is 9.3. The enzyme seems to be introduced in the invading osteoblasts for it is absent in cartilage before ossification.

[2] Rickets is also known as rachitis (Greek *rhachis*, the boll of a tree, and hence applied to the spinal column, originally supposed to be the seat of the chief lesion in rickets). Tabes (Greek *teko*, to melt; hence applied to the disappearance of bone, nerves, etc.). For Rickets see page 643.

[3] While rickets may affect the adult, it is seldom encountered.

[4] Page 215. See also page 645 for a more complete treatment of rickets.

[5] Greek *akros*, top, and *megas*, great; signifying the enlargement of the extremities. See page 667.

pituitary[1] becomes diseased, due to some functional disturbance or to pressure. In craniotabes, bone is lacking in the phosphate. In chondrodystrophia (achondroplasia) the epiphyses of the long bones ossify precociously while the shafts remain unossified until later; growth is inhibited and a dwarf results.

Bone-marrow is a vascular, fatty[2] substance containing hematopoietic[3] organs in which the erythrocytes, or red blood-corpuscles, are made. The substances found in blood are also to be found in the marrow and there is nothing especially characteristic about this substance from the chemical standpoint. The "bone oil" which attracted attention among some of the older chemists, like Glauber, contains complex aromatic substances, like pyrrols; these substances are derived from amino-acids in bone. From the mesenchyme cells, that invade the developing bone, are formed the parents of the two groups of cells, namely, the histiocytes which form the endothelial walls of the bone-marrow sinuses and secondly the erythroblasts which form the white and red cells. All of these cells compose the marrow.

Exercise 1.—A sheep rib has been soaked in dilute acid solution for a week. Secure 25 mls. of the solution and about 3 cms. of the residual bone. Save[4] it for later study. Follow the directions (page 359ff.) for the study of the solution, using 25 mls. of fluid for the detection of inorganic cations and anions:

Inorganic Constituents:[5]

Anions: (1) *Chlorion*,—Cl^-.—Place 2 mls. of the fluid in a test-tube and add 1 drop concentrated nitric acid, HNO_3, and 1 ml. 2 per cent. silver nitrate, $AgNO_3$, solution.[6] A positive test is obtained if there is a whitish precipitate of silver chlorid which redissolves on the addition of ammonium hydroxid. Small amounts of silver chlorid cause only a cloudiness in the solution. Confirm the test by scraping a piece of dried bone until you have accumulated as much as a 5-cent

[1] In acromegaly the anterior lobe of the pituitary gland is affected. See page 667.

[2] In necrotic conditions, such as senescence, or old age, there is a chondroid marrow which is not fat-like in constitution.

[3] Greek *'aima*, blood, and *poiein*, to make.

[4] Page 361.

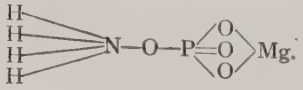
[5] The study is to be made without chemical separation, such as the analytical chemist usually uses. If time permit, it is better to proceed as in qualitative analysis, using the usual procedures found in any good qualitative analysis, such as Treadwell-Hall.

[6] This is practically a decinormal solution, which contains 1.7 per cent. $AgNO_3$; the decinormal solution made for chlorid analysis may be used.

piece will hold. Transfer the scrapings to a mortar containing 1 volume of quicklime, CaO, and grind the substances until thoroughly mixed. Transfer the substance to a small porcelain crucible and place on a pipe-stem triangle over a Bunsen burner. Cover with a porcelain crucible cover and leave a small opening for the escape of steam and fumes. Heat gently at first and then strongly to fusion. Remove the flame, permit to cool in the air, add distilled water and a drop or two of concentrated nitric acid. Then add silver nitrate solution as before as long as there is a precipitate. The whitish precipitate indicates the presence of a halogen.

(2) *Phosphion*, $-PO^{-}_{4}$.—(*a*) The solution: To 5 mls. of the solution add NaOH solution until neutral to litmus. Then add 1 volume concentrated sulphuric acid, H_2SO_4, and 1 volume nitric acid, HNO_3. Clamp the tube in a slightly inclined position in the hood and heat gently with a Bunsen burner until color has been lost. If the color is not lost, cool under the tap and add an additional 1 ml. of concentrated HNO_3; heat again, as before. Now permit to cool and continue cooling under the cold-water tap. Add 2 mls. Benedict's molybdic acid reagent[1] and 2 mls. of Benedict's hydrochinon reagent (page 891). The presence of a blue color indicates the presence of phosphorus.

(*b*) Scraped bone: Place about 2 gs. of the scrapings in a porcelain crucible and mix thoroughly with 3 volumes of "fusion mixture"[2] by means of a stirring-rod. Place the crucible on a pipe-stem triangle resting on a tripod over a Bunsen burner. Fuse as described for chlorion above[3] and extract the mass with cold water. To the solution add an excess of concentrated ammonium hydroxid and then drop-by-drop "magnesia mixture."[4] Phosphates are precipitated as ammonio-magnesium phosphate,



(3) *Carbonate* ion, $-CO_3^{=}$.—Add a drop of concentrated HCl to dry bone substance, effervescence of CO_2 occurs, indicating the presence of the radicle $-CO_3^{=}$.

Cations.—(1) *Sodion*, Na^{+}.—Sodium imparts a yellow color to the flame of a Bunsen burner. Demonstrate the presence of sodion in

[1] Page 891.

[2] Appendix. The mixing should be done by grinding the ingredients in a mortar.

[3] Page 359.

[4] Appendix.

the solution by igniting a small shred of long-fibre asbestos until a yellow flame no longer appears; then dip the cooled asbestos in the solution to be tested and heat again in the flame; a yellow color appears.

(2) *Potassion*, K^+.—To 5 mls. of the solution add 10 per cent. NaOH solution to neutrality to litmus paper. Acidulate with 2 mls. glacial acetic acid and boil to remove the ammonia. Cool. Then add 2 mls. sodium cobalti-nitrite solution[1] and let stand for fifteen minutes or longer. A positive test is indicated by the appearance of a yellow precipitate of potassium-sodium-cobalti-nitrite, $K_2NaCo(NO_2)_6$. There must be more than 0.3 mg. potassium present to give a distinct test.

(3) *Calcion*, Ca^{++}.—Since other alkaline earth elements, like barium and strontium, are not present in bone, one may proceed directly to the detection of calcium in bone: Add to 1 volume of the solution to be tested 1 volume of ammonium oxalate[2] solution, $\begin{matrix}COO.NH_4\\ |\\ COO.NH_4\end{matrix}$; in the presence of calcion insoluble calcium oxalate[3] appears as a white precipitate, $\begin{matrix}COO\\ \diagup\\ \diagdown\\ COO\end{matrix}\!\!>\!Ca$.

(4) *Magnesion*, Mg^{++}.—It is necessary to remove all heavy and alkaline earth cations before testing for magnesium: To 5 mls. of the solution add concentrated ammonium hydroxid to a slight alkalinity; filter. Save the filtrate and to it add half its volume of 5 per cent. ammonium carbonate solution; filter again. Then to the filtrate add a few drops of concentrated ammonium hydroxid and a few drops of 5 per cent. di-sodium monohydrogen phosphate, Na_2HPO_4, solution. A positive test is indicated when a white, crystalline precipitate of ammonio-magnesium phosphate appears, according to the following reaction: $Mg^{++} + Na_2HPO_4 + NH_4OH \longrightarrow MgNH_4PO_4\downarrow^4 + 2Na^+ + H_2O$.

The Organic Part.—The chemistry of "ossein" is similar to that of collagen. The specimen of bone given you contains mucoid and albumoid, in addition to collagen. Leave the demineralized piece of bone in lime-water[5] until the following period to extract the mucoid

[1] Appendix. The substance is $Na_3Co(NO_2)_6$.

[2] Appendix.

[3] Solubility of calcium oxalate in H_2O at room temperature is 0.000,056 g. per 100 mls. H_2O.

[4] The inverted arrow indicates that the substance is precipitated.

[5] Appendix. A half-saturated solution is used.

and albumoid.[1] Then remove it and test by the following methods for protid:

(1) Using 5 mls. of the solution, which is alkaline, acidulate with glacial acetic acid to neutrality to litmus paper and then add 1 drop more to give acid reaction. Perform the heat-coagulation test[2] and note that coagulation occurs. Save the filtrate from this coagulated mass. Try Millon's test and the biuret reaction.

(2) Using the filtrate from (1), perform the biuret reaction upon a portion of it and, upon a second part, determine whether the Hopkins-Cole reaction (page 312) is positive or negative; a positive test indicates the presence of the so-called "albumoid."

(3) Using the collagen left in the bone after removal of these two organic substances, repeat the tests given on page 312.

Muscle.—The chief function of muscle is to shorten (contract). How this is accomplished can be considered to better advantage after the chemistry of muscle has been discussed.[3] Of the two kinds of muscles, voluntary and involuntary, or smooth muscle, the latter is less specialized. It is composed of simple elongated cells, each containing a single nucleus. On the other hand, striated muscle consists of syncytia[4] or pseudo-cells having one cell-wall in common and several nuclei. The sarcolemma,[5] which seems to be the seat of the actual contracting of the muscle, lies peripherally to the nuclei, and it is probable that this sarcolemma is a thickened and specialized portion of the cytoplasm. It is this substance which gives rise to the appearance of cross and longitudinal striations. While there has been much discussion concerning the intimate structure, it is more than probable that the horizontal striations of the cell are due to alternate zones of contracting and lax substances. As micro-dissections have shown when the sarcolemma is pulled about by means of a needle, these striations disappear. The striations are, therefore, to be likened to waves in the continuous plasma of the cells; the more dense portions form the crests and the less dense ones the troughs of the waves. The polariscope shows that these portions affect light, which has been

[1] If desired, the osseomucoid (see Hawk, P. B. and Gies, W. J., Amer. Jour. Physiol., vol. 5, page 387, 1901) may be studied by decanting the lime-water, filtering, HCl added as long as a precipitate forms. Purify the precipitate by redissolving in 1 per cent. Na_2CO_3 and then reprecipitate with HCl. Wash by decantation with 0.1 per cent. HCl, then with distilled H_2O and finally with 85 per cent. ethanol. The chief component is chondroitin sulphuric acid.

[2] Page 763.

[3] Page 379.

[4] Greek *syn*, together, and *kytos*, a cell.

[5] Greek, *sarx*, flesh, and *lemma*, covering or husk.

polarized,[1] differently: The denser portions appear light and hence are anisotropic,[2] while the lighter portions are isotropic, that is, emitting only one ray. Since double refraction may be caused artificially by stress or strain in the substances exhibiting double refraction, it is probable that the denser portion is under stress (contraction), while the less dense substance is in equilibrium. Such are the observational features of muscle contraction.

Exercise 2.—Concentrate the extract from the hydrolysis experiment of page 235, or use commercial meat extract, like Steero, or Bovril for the following separation of the "extractives" of muscle:

(A) Dilute 20 mls. of the solution from the earlier experiment with 100 mls. of distilled water, or dissolved 10 gs. of commercial extract in 100 mls. of hot water. Add, in small portions, as the precipitation of inorganic substances takes place, lead acetate solution (9 per cent.) as long as a distinct precipitate occurs, avoiding an excess of the reagent, which has to be removed. Permit the precipitate to settle and then decant the supernatant fluid into a test-tube; pass a current of hydrogen sulphide[3] through it for two minutes. To be certain that all of the lead has been removed, filter off the lead sulphide, return the test-tube to the generator, and pass the gas through again; if it has not, let the current of gas run through for two minutes longer and filter again. Transfer the clear filtrate to a small evaporating dish and, carefully, by means of the micro-burner, evaporate off the excess liquid, leaving a syrup. Let this stand protected by a piece of filter-paper until the following period. Crystals of creatin will appear and they should be examined under a microscope with low and high power. Dilute the desk reagent of ethanol (95 per cent.) to make 50 mls. of 88 per cent. ethanol solution[4] and transfer the crystals to this solution. Stir to insure complete mixture and solution of irrelevant substances, which are removed by filtration, the

[1] Page 183.

[2] Greek *an*, privitive, meaning without, *isos*, same, and *tropos*, turning, that is, "not turning the same way;" the substance is "doubly refractive," that is, there is an ordinary and extraordinary ray sent out by the denser portion, which corresponds to a crystal of iceland spar (page 186), and the reason the denser portion looks lighter in the polariscope is because one or the other ray passes through the instrument whatever the position of the polariscope prisms with respect to one another. On the other hand, the isotropic less dense portion of the muscle protoplasm is dark when viewed by the polariscope when the prisms are "crossed," that is, the plane of polarized light is 90 degrees from the plane of the analyzer.

[3] Appendix.

[4] Table in Appendix.

crystals of creatin being insoluble in this strength alcohol. They may be purified by washing them while on the paper with 88 per cent. alcohol and then dissolving them in hot water. Add a small amount of good charcoal, mix, and filter while hot. The filtrate should be colorless. Transfer the filtrate to a boiling water-bath and concentrate to about 25 mls. solution. Let cool; white crystals of creatin appear.

The Chemistry of Creatin. The reader should refer to page 259 where the formula for guanidin is given in connection with the diamino-acid arginin. Guanidin is $H_2N-\overset{NH_2}{\overset{|}{C}}=N-H$. Creatin is alpha-methyl-guanidin-acetic acid. Methyl guanidin is derived from guanidin by the replacement of a hydrogen belonging to one of the two amino-radicles, NH_2, by a methyl group, CH_3, as follows:

$$\underset{\text{Guanidin.}}{H_2N-\overset{H-N-H}{\overset{|}{C}}=N-H} + \underset{\substack{\text{Methyl}\\ \text{radicle.}}}{CH_3} \longrightarrow \underset{\text{Methyl-guanidin.}}{H_2N-\overset{H-N-CH_3}{\overset{|}{C}}=N-H}$$

Creatin is produced when the second hydrogen of the same nitrogen is replaced by acetic acid, CH_3COOH. The process is identical with the first one of methylation, for the methyl group of the acetic acid replaces the hydrogen from the nitrogen radicle:

$$\underset{\text{Methyl-guanidin-acetic acid, or creatin.}}{\begin{matrix} HOOC.H_2C-N-CH_3 \\ | \\ H_2N-C=N-H \end{matrix}}$$

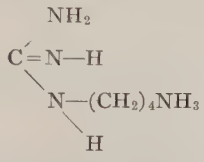
Methyl-guanidin and Pathological States.—Methyl-guanidin has been known for some time to be toxic; it is detoxicated by the process of acetylation to form creatin. Di-methyl-guanidin is the cause of tetany:

$$\begin{matrix} & & NH_2 & & \\ & \diagup & & & \\ C & = & N-H & & \\ \diagdown & & & \diagup & CH_3 \\ & & N & & \\ & & & \diagdown & CH_3 \end{matrix}$$

It is a question whether the tetany that follows removal of the parathyroid is due to guanidin intoxication or not. Collip believes that they are not identical, Looney finds in cases of muscle rigidity (catatonic precox) an increase in blood creatin and a decrease in blood calcium; on the administration of Collip's parathyroid extract, the rigidity is lessened, and the calcium rises. This is in accord

with the earlier work of Noël Paton, Sharp and others, who contend that guanidin is responsible for tetany. When methyl guanidin is administered to an experimental animal, tetany and loss of calcium occur.

Recently the earlier work of Underhill on the relation between blood sugar and tetany has been reinvestigated. Di-methyl-guanidin causes convulsions of tetany regardless of the height of the blood sugar. In order to differentiate the toxic principle from that affecting blood sugar, Frank[1] and co-workers studied different guanidin compounds, first, agmatin the butylamin of arginin (page 258).



which in nontoxic doses depresses the blood sugar 30 per cent. Aminopenthylen-guanidin containing one more methyl group gives an effect similar to that of insulin, an effect which is counteracted by administering glucose. Higher members of this series have been synthesized, and one of them, *synthaline*, has been selected as a partial substitute for insulin. The maximum dose permits about 45 grams of glucose to be metabolized. The action is less immediate, but more lasting than that of insulin. Administration is made by mouth. Owing to its slow action it is of little avail in combatting diabetic coma. The action of guanidin preparations on sugar have been investigated by Witzemann,[2] who finds a combination of guanidin and glucose in which the properties of glucose are fundamentally altered.

A colorimetric method for methyl-guanidin has been devised by Pfiffner and Myers.[3]

Creatin, Normal and Pathological.—Normal: Creatin occurs in various organs of the body in varying amounts. The following table from Andrew Hunter[4] gives the distribution in some tissues of man and other animals:

[1] Frank, E., Nothmann, M. and Wagner, A., (Berliner) Klinische Wochenschr., vol. 5, page 2100, 1926.

[2] Witzemann, E. J. (Rush Medical College, Chicago) Journ. Amer. Chem. Soc., vol. 46, page 790, 1924.

[3] Pfiffner, J. J. and Myers, V. C. (page 806) Proc. Soc. Exp. Biol. and Med., vol. 23, page 830, 1926.

[4] Hunter, Andrew (contemporary), Professor of Biochemistry, University of Toronto, Canada. See Physiol. Reviews, vol. 2, p. 590, 1922.

	Mgs. per 100 mls. substance.
Human striated muscle (adult)	393
Human striated muscle (child, 1.75 year)	331
Human striated muscle (at birth)	190
Cardiac muscle of beef	240
Smooth muscle (human uterus)	52
Smooth muscle (human uterus) at delivery	89
Smooth muscle (human uterus) after delivery	51
Brain of dog	108
Testis of dog	181
Liver of dog	25
Pancreas of beef	15
Kidney of beef	18
Spleen of dog	20
Blood of dog	20
Blood of man	2.1–4.9
Milk (human)	3.0

Ninety-eight per cent. of the total body creatin occurs in muscles, and especially in voluntary muscles, probably in combination with phosphoric acid.[1] Of the remaining 2 per cent., three-quarters are found in the brain. In the young, creatin occurs in small quantities and the amount increases with age. However, the proportion of content of creatin to volume of musculature is greater in the child than in the adult. The uterus shows varying amounts of creatin which are correlated with the activity of that organ. In general, one may conclude that creatin is an index of the degree of activity of the muscle.

Creatin appears normally in the urine of children of both sexes, owing to the larger content of this substance in the child in proportion to the volume of musculature. Its excretion appears longer in the female child than in the male. After puberty creatin is abnormal in the urine of the male, but is normal for the female during the menstrual periods, the menopause,[2] pregnancy and especially immediately after parturition.[3] Pathologically, creatin is increased in the urine or appears in the urine during starvation, febrile[4] conditions, diabetes mellitus, high protid diet, and attendant states, such as low glucid diet,[5] exophthalmic goiter,[5] etc.

[1] Page 732.

[2] Greek, *men*, lunar month, and *pauo*, to restrain; the cessation of the monthly or menstrual periods occurring at about forty-five years in the American woman.

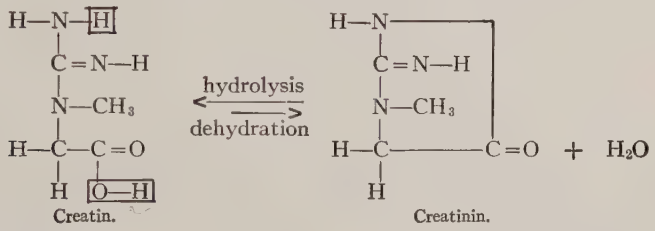
[3] Latin *pario*, to bear children; child-birth.

[4] Fever.

[5] Low glucid diet means excessive metabolism of protid, since glucid is a protid sparer (page 566). In exophthalmic goiter metabolism is increased; this is used as a means of detecting the presence of the disease (page 630).

The Origin of Creatin. We have called attention to the fact that the guanidin radicle appears in the amino-acid, arginin, but on feeding this substance, under ordinary circumstances, creatin is not increased in the urine. However, if the feeding is made while muscles are actively exercising, arginin is utilized and creatin appears in the urine. The Jaffé-Paton[1] detoxication theory for the origin of creatin holds that since methyl-guanidin and guanidin are toxic, they may be converted into methyl-guanidin-acetic acid (creatin) and thus become detoxicated. Other possible sources of creatin have been suggested, but at present no theory bears the scrutiny of experiment.[2]

The Anhydrid, Creatinin.—If creatin be dehydrated by being boiled with alkali or acid, the following reaction occurs:



Note the sign of reversibility in the above reaction; equilibrium is reached farther on the creatin side of this equation during acid hydrolysis and this is the usual procedure.[3]

Exercise 3.—Using purified creatin, obtained from Exercise 2 above, apply the picric acid reaction of Jaffé to a solution of a few crystals as follows: Place about 5 mls. of the creatin solution in a test-tube and add one volume of alkaline picrate solution.[4] Mix. Set the test-tube aside in your rack and proceed with the following reaction: Into a second test-tube place 5 mls. of the creatin solution and add 1 volume of 10 per cent. HCl solution, or an equivalent amount of concentrated hydrochloric acid. Boil in a boiling water-

[1] Jaffé, Max, German biochemist, died 1911, responsible for the chief qualitative test for creatin (pages 367, 735), by way of the anhydrid, creatinin. D. Noël Paton (page 260).

[2] Hunter gives the following relations of the conversion of creatin into creatinin: In decinormal alkali at 20° C., after two days, only 0.1 per cent. creatinin occurs, 0.9 per cent. being creatin. Hence, in alkaline hydrolysis, less creatinin is obtained, since the equilibrium is reached farther on the left side of the equation, that is, the creatin side.

[3] Appendix.

[4] Mann (page 474) has found that extirpation of the liver does not affect the creatin-creatinin metabolism.

bath for ten minutes or longer. Neutralize to litmus by adding 10 per cent. NaOH solution and add in addition about 1 ml. of the alkali.[1] Now add one volume of the alkaline picrate solution used in the first tube; compare the colors in the two tubes. The reaction is due to the formation of creatinin picrate which has been isolated by Greenwald.[2]

EXERCISE 4.—*Weyl's*[3] *Test with Nitro-ferro-cyanid Solution.*—Grind upon a piece of paper or upon your desk-top by means of your thumb-nail two small crystals of sodium nitroferrocyanid (sodium nitro-prussid, $Na_2Fe(CN)_5NO.2H_2O$) and transfer the powder to a test-tube. Acidulate with a few drops of acetic acid, or add 5 mls. of distilled water to the crystal and then add 1 drop of glacial acetic acid. Then add 1 volume of the creatin-creatinin solution after hydrolyzing as described in Exercise 3. Neutralize with 10 per cent. NaOH, then layer with NH_4OH or with 10 per cent. KOH or NaOH. A color somewhat similar to that of the crystals of nitroferrocyanid is obtained at the zone of contact of the two points.

Creatinin in the Urine.—While we shall study the urine in detail later (Chapter XV), it is well to call attention to the fact that such a substance as the anhydrid of creatin of muscle and blood appears normally in the urine. This is demonstrated as follows:

EXERCISE 5.—Obtain a specimen of your urine. Place 5 mls. of it in a test-tube. Acidify with a drop of glacial acetic acid and add some powder from a crystal of sodium nitroferrocyanid as in the above test of Weyl. Mix well and overlay with concentrated ammonium hydroxid. A ruby ring indicates the presence of creatinin.[4] The reaction is similar to that of secondary amins[5] treated with

[1] In place of using NaOH one may use only enough NaOH to neutralize, then add the solution to be tested, and layer carefully with the NH_4OH; color appears at the contact.

[2] Page 23, note 5.

[3] Weyl, T., German chemist, died 1913.

[4] This same reaction is given by the Legal test for aceton and indol, but in the case of aceton the addition of glacial acetic acid accentuates the ruby-red color and, if there is no aceton, a straw yellow color develops. For indol, the addition of the acid causes the appearance of a bluish color, the depth being determined by the concentration of the indol. (Legal, see his test for aceton in Chapter XV.)

[5] A secondary amin is one in which two of the hydrogens of ammonia are replaced by another element or radicle:

$$\begin{array}{c} H \\ | \\ H-N-H \\ | \\ H \end{array} + 2R = \begin{array}{c} R \\ | \\ R-N-R. \\ | \\ H \end{array}$$

nitrous acid, where a nitroso-compound results.[1] These are yellowish compounds, and in all of the above reactions involving sodium nitroferrocyanid the yellow appears after standing.

EXERCISE 6.—Using your urine as in the previous Exercise, repeat Jaffé's test given above in Exercise 3, page 367. This test is the basis of the Folin quantitative method for creatin and creatinin.[2]

The Physiology and Pathology of Creatinin.—Although we do not know the relations of creatin and creatinin in the human body, the simple chemical relation suggests that somewhere creatin gives rise to creatinin. No really satisfactory method for the quantitative study of creatin in small amounts exists. For larger amounts the Folin method, by way of creatinin, is excellent. Benedict[3] has pointed out that there is abundant chance for error where small quantities of either creatin or creatinin are involved. Hence determinations of creatin and creatinin in blood and tissues must not be accepted as final. Creatinin has been reported from muscle tissue in amounts of from 1.6 to 134 mgs. per 100 gs. of muscle tissue; in sheep testis, from 209–215 mgs. per cent. and from 0.1 to 3 mgs. per cent. in human blood.[4] Caution must be exercised in generalizing concerning these substances.

Regarding the grosser amounts of creatinin, however, certain information has been acquired. The question may be asked, What rôle does it play in the body? It is excreted into the urine in definite amounts varying with different persons, but, as Folin[5] has shown, constant for each individual. It must, therefore, possess an important function. Folin described it as an "endogenous substance" which is one that concerns the metabolism of the body proper as contrasted with the metabolism of the food materials; that is, it represents an end-product of some process of body transformation. The main reasons for believing this to be its function are: (1) that it

[1] For example, dimethyl-amin, treated with nitrous acid:

Dimethylamin. Nitrous acid. Nitrosamin.

[2] Page 735.

[3] Behre, J. A., and Benedict, S. R., Jour. Biol. Chem., vol. 52, p. 11, 1912.

[4] Chapter XVI. From Benedict's work it appears that only a part of the "creatinin" ordinarily considered as creatinin in blood can be true creatinin.

[5] Folin, O. (page 19), Amer. Jour. Physiol., vol. 13, p. 66, 1905.

is excreted almost quantitatively when fed;[1] (2) that it is not excreted in larger quantities when protid or other food is increased in the diet; and (3) that it is excreted uniformly not only per day, as Folin has shown, but hourly, as Shaffer[2] found. The fairly constant relation between the creatin content of muscle and the excretion of creatinin suggests that creatinin represents an index of muscle metabolism. Further discussion of creatin and creatinin will be made.[3]

The Purins of Muscle.—The chemistry of the purins of muscle extract has already been discussed.[4] These substances are derived from the purins of the nucleus, the amino-purins adenin and guanin, by oxidation; from adenin hypoxanthin is obtained, and from guanin, xanthin; lastly, uric acid comes from the oxidation of xanthin:

Hypoxanthin..... $C_5H_4N_4O$

↓

Xanthin..... $C_5H_4N_4O_2$

↓

Uric acid..... $C_5H_4N_4O_3$.

The reason why the oxypurins are found in muscle extract in amounts of about 0.1 g. per 100 gs. of substance is because no enzyme exists in muscle capable of oxidizing them farther. Such an enzyme does occur in liver[5] where these oxypurins are farther oxidized to uric acid. In muscle no demonstration, free from criticism, has been made of muscle purin metabolism which results in an increase in purin excretion after muscular exercise; but experimentally it has been shown that there is an increase in purins when a muscle is exercised after being removed from the body and suspended in isotonic saline,[6] the fluid surrounding the muscle being analyzed for purins. A small amount of uric acid has been found in such cases, but this may have been derived from included blood, which maintains a constant level of uric acid of about 0.004 g. for 100 mls. of human blood.

[1] About 10 to 20 per cent. of creatinin fed is retained for a time in the body, probably because the reaction creatin $\rightleftharpoons$ creatinin is reversible, and in alkaline media, like the body fluids, over twice as much creatin remains in solution after twenty-four hours as creatinin, since the equilibrium constant, K, in the above reaction is 2.12 (Hunter, p. 588).

[2] Shaffer, P. A. (Washington University, St. Louis, Missouri). Fig. 163. See Amer. Jour. Physiol., vol. 23, p. 1, 1908. The range varies from day to day to the extent of 10 per cent.

[3] Chapters XV and XVI.

[4] Page 331.

[5] Page 339.

[6] Page 122.

Pathological States and Purin Metabolism of Muscles.—There is a general increase in nitrogenous substances derived from muscle in those diseases involving excess activity of muscles. Mere increased muscular action does not lead to an increase in muscle purins, but fever is accompanied by excessive excretion of all nitrogenous substances, or their products, derived from muscle.[1] Muscular atrophy likewise is accompanied by heightening purin excretion, as it is by the excretion of "extractives" in general.

Exercise 7.—Heat to boiling in a 500-ml. beaker 250 mls. of distilled water, and in this dissolve 25 gs. of meat extract. Repeat the precipitation of protid, etc., as given for creatin[2] previously, by adding lead acetate solution until no further precipitation occurs. Leave ten minutes or longer. Filter, first through cotton or glass-wool, then through paper, while hot, saving only the filtrate. Pass hydrogen sulphide[3] through it from a generator to precipitate the lead as lead sulphide. Filter again. Place the filtrate in an evaporating dish on the steam-bath in the hood and leave until evaporation has been completed. Dissolve the residue in 150 mls. of 88 per cent. ethanol[3] and filter. Discard the residue and transfer the filtrate to an evaporating dish. Leave the dish on the bath until all odor of alcohol has been removed; the volume now should be about 100 mls. To it add 2 mls. concentrated NH_4OH, and then, carefully, drop by drop, ammoniacal silver nitrate solution[3] as long as precipitation of hypoxanthin-$AgNO_3$ and xanthin-$AgNO_3$ occurs, avoiding excess. Filter, wash once with cold distilled water, and save the residue. Carefully remove the filter-paper containing the hypoxanthin and xanthin silver salts and place it in an evaporating dish. Convert the silver compounds into nitrates by adding enough nitric acid solution[4] to cover the paper, boil for about one minute, and filter while hot. Wash with a small amount of the diluted nitric acid solution. Permit the filtrate to cool, whereupon crystals of hypoxanthin-silver nitrate appear. Let stand until the following period; then filter,[5] wash with 50 mls. of distilled water, and save both filtrate and residue.

[1] Note the relation of creatin and creatinin excretion to febrile conditions mentioned previously on page 366.

[2] Page 363. [3] Appendix.

[4] Concentrated HNO_3, 2 volumes; distilled water, 3 volumes. Boil the solution to remove any nitrous acid that may be present; it is capable of deaminizing the purins (*cf.* page 294).

[5] It may be necessary to use two of the ordinary filter-papers to hold the precipitation.

Residue: Transfer the residue by means of a spatula or knife to a small beaker. Add 25 mls. distilled water and pass a current of hydrogen sulphide from a generator through the liquid. Filter off the silver sulphide which is formed, leaving in the filtrate hypoxanthin nitrate. Study the crystals. Hypoxanthin may be obtained from the nitrate as follows: Bring the solution to 110° C. to drive off the excess H_2S; make ammoniacal with a few drops of concentrated NH_4OH; filter; concentrate the filtrate on the water-bath until one-fourth of the liquid has evaporated and then let stand. The crystals of hypoxanthin which separate with some difficulty are small and the higher power of the microscope must be used in their identification.

Filtrate: To the filtrate obtained from the residue just studied add 5 mls. concentrated ammonium hydroxid. Filter, saving the residue. Wash this residue into a small beaker with about 20 mls. distilled water and pass a current of H_2S through the cold solution for not over one minute. Bring to about 80° C., filter, and permit to stand in a cool place until crystals of xanthin-nitrate appear. From the nitrate crystals of xanthin may be secured by adding a few drops of ammonium hydroxid and permitting the solution to concentrate slowly on the water-bath at not over 50° C.

Qualitative Tests for Oxypurins: (1) *Hypoxanthin.*—Make slightly alkaline with a drop of 10 per cent. NaOH solution a suspension of crystals of hypoxanthin (or as salt), and then add a drop or two of a solution of the reagent di-azo-benzene-sulphonic acid;[1] a red color develops unless there has been too much alkali added. This reaction is given also by xanthin. Hypoxanthin may be distinguished from xanthin by the failure to give the reaction as follows: Add to the hypoxanthin suspension 1 volume of concentrated HNO_3 and evaporate to dryness on a water-bath at 100° C. If xanthin is present a yellow residue results which turns reddish when cold 10 per cent. NaOH solution is added and purple when heat is applied. With hypoxanthin no colors, save a yellowish tinge due to some nitrous oxid, will appear.

(2) *Xanthin.*—Repeat the two reactions given above, using suspensions of xanthin or the nitrate. In addition to these two, perform the following reaction: To a few crystals suspended in water in an evaporating dish add one volume of freshly made chlorin water,[2] and boil to dryness first over a free flame and then on a boiling water-bath. To the whitish or yellowish residue bring the wetted stopper of the

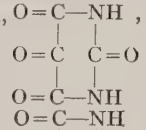
[1] Sometimes called diazotized sulphanilic acid: $C_6H_5N = NOSO_2OH$.

[2] Appendix.

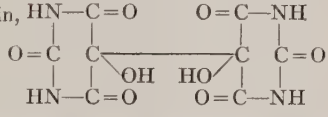
reagent bottle containing concentrated NH_4OH; a reddish color results, due to the formation of alloxantine-ammonia, or murexid.[1]

The *mononucleotid, inosinic acid*,[2] has been mentioned before. It occurs in muscle and was formerly known as carnine. Several other substances affiliated with inosinic acid have been isolated from meat extract, such as carnitin, phosphocarnic acid, etc. Carnosin, probably derived from the amino-acid histidin, likewise occurs. It is of theoretical interest in that it gives, on acid hydrolysis, a beta-amino-acid, beta-alanin, making therefore an exception to the statement that all amino-acids occurring in the organism are alpha-compounds, but it has never been shown that carnosin is an integral part of muscle. A betaïn, carnitin,[3] occurs, but its significance is unknown It belongs to the group of natural bases, which include such compounds as cholin, neurin, muscarin, etc. It is possible that it is derived from, or else is a precursor of, cholin. However, we have several muscle substances like inosinic acid which are more or less immediately

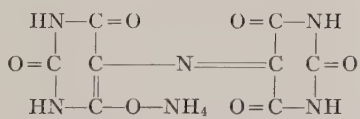
[1] So named from the sea-shell Murex, from which the ancients, particularly the Romans, secured this compound and called it "Tyrrhean Purple." This name, however, according to Friedländer (Ber. d. d. chem. ges., vol. 42, page 765, 1909) should not be applied to murexid because the Purple is a di-brom-indigo. The murexid reaction consists in the oxidation of xanthin into uric acid, and this to alloxan,



which, in turn, becomes condensed to alloxantin,



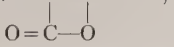
. On adding the ammonium hydroxid, alloxantin becomes the imino-compound in place of the hydroxy-form:



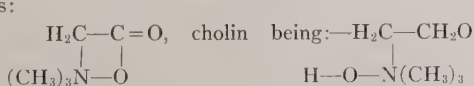
Hypoxanthin does not give this reaction because it cannot be oxidized to xanthin under the conditions of the experiment.

[2] Page 339.

[3] The betaïns are typical plant products, so-called because of the extraction of this compound from the juice of the sugar-beet, Beta vulgaris, which is the source of much of our sugar. The structure of betaïn is:

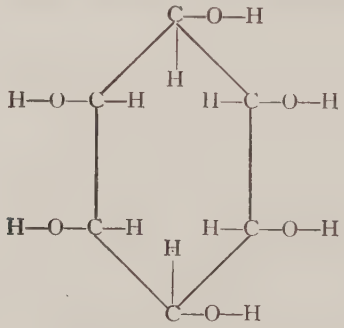


and the resemblance to cholin which occurs in lecithin (page 210) may be brought out by rearranging the formula as follows:



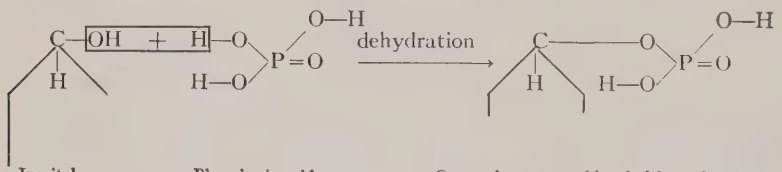
derived from plant food material.[1] Carnosin responds to the diazo-reaction given above for xanthin bases (page 372), and this reaction has been made the basis of a quantitative method for carnosin by George Hunter.[2]

Inositol ("muscle sugar") is not a sugar, although it has the empirical formula of the hexoses—$C_6H_{12}O_6$. Its real structure is rather that of a saturated benzene ring,[3] the structural formula being:



(Hex-hydroxy-hydro-benzene, or inositol).

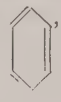
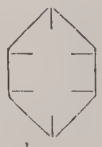
Here again we seem to find a substance which is derived from the plant foods, for a close chemical relationship holds between inositol of muscle and phytin of the plant. Phytin is the calcium-magnesium salt of inositol-hexaphosphoric acid; each carbon atom in inositol is united in ester formation with phosphoric acid, H_3PO_4, as follows:



Inositol. Phosphoric acid. One carbon-atom of inositol-hexaphosphoric acid.

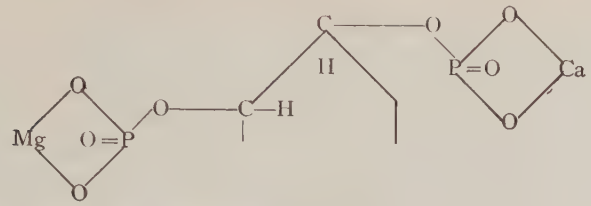
[1] Inosinic acid contains a pentose, ribose, which is not found in any animal nuclear material derived from the chromatin of the cell. Since pentoses are characteristic of plants, it is supposed that these are the source of inosinic acid, that is, a plant mono-nucleotid. Similarly, carnitin is more typical of plant substances than animal material.

[2] Hunter, G. (English biochemist). See Biochem. Jour., vol. 15, p. 689, 1921. Compare the colorimetric method for iminazoles by Koessler and Hanke, Jour. Biol. Chem., vol. 39, p. 497, 1919.

[3] The benzene ring, as conceived by Kekulé, the German chemist who first used the configuration, is unsaturated and is usually written thus:  , or thus: 

so that each carbon atom will have its full number of bonds (four). In benzene each carbon carries one hydrogen atom.

Calcium and magnesium are attached, each replacing two hydrogens, thus:



Two carbons of inositol-hexaphosphoric acid with magnesium and calcium to form phytin.

In the above scheme only two of the six carbon atoms of the aromatic ring are shown, carbons 1 and 6. Since inositol is not easily obtained from muscle extract,[1] it is assumed that it is in chemical union with some substance in the muscle. The structure of phytin may give the key to its condition in the muscle, but if inositol has any special function in the tissue, we are ignorant of its nature. It may be possible that it is combined with phosphoric acid and creatin. (J.M.L.). While its empirical formula might suggest a relationship to sugar, it is quite certain that no such relation exists.[2]

Glycogen (animal starch[3]) occurs in muscle to varying extents, the average being about 0.4 g. per 100 gs. of human muscle. It is greater in amount in a "trained" than in an "untrained" muscle (Embden). It is readily converted into glucose and hence muscle must be treated by some method in order to kill the enzyme responsible for the conversion of glycogen into glucose. This may be done by the method used in a former Exercise, in which glycogen was obtained from the liver,[4] that is, by plunging the muscle into boiling water immediately after removal from the body. Glycogen may be removed quantitatively from the muscle by feeding an experimental animal phlorhizin,[5] an agent which prevents the kidney from reabsorbing[6] sugar and hence imitating diabetes mellitus. Or it may be

[1] A small amount of inositol is undoubtedly free in muscle.

[2] Two reasons have been given for believing that inositol has some relationship with the glucids, namely, (1) furfural may be obtained from it (page 167) and (2) lactic acid appears in the urine of animals that have been fed inositol. Furfural is a typical product of 5 and not of 6 carbon chains; moreover, since inosite produces profound effects when fed experimentally, such as diarrhea, it may interfere with the normal oxidation of either the fats or glucids, thus causing the excretion of lactic acid.

[3] Page 190.

[4] Page 191.

[5] Chapter XVII.

[6] Benedict believes that phlorhizin prevents the kidney tubules from reabsorbing glucose which is excreted through the glomerulus. See Nash, T. P., and Benedict, S. R., Jour. Biol. Chem., vol. 55, p. 757, 1923.

removed by the piqûre operation of Claude Bernard,[1] which consists in passing an instrument something like a hat-pin through the occiput of an experimental animal, directing it downward and somewhat forward through the medulla of the brain, near the floor of the fourth ventricle, where it strikes the so-called "sugar center." This, when irritated,[2] causes a mobilization of all glycogen; there is a sudden hyperglycemia and glycosuria. It must be observed that this is not a true diabetes mellitus,[3] for there seems to be no effect upon the hormon of the pancreas. Similarly, phlorhizin glycosuria is not a true diabetes mellitus, for, as Benedict[4] and co-workers have shown, the effect of phlorhizin is on the kidney and not on the pancreas; it is of the nature of "renal" glycosuria.[5] In either case the glucose is drained into the urine and glycogen is called upon to restore the loss. Immediately after the cessation of flow of blood through the muscle glycogen is hydrolyzed to glucose, probably owing to a change of reaction of the tissues and blood from the normal alkalinity to slight acidity.

Lactic acid in the form of "sarco-lactic acid,"[6] chemically designated as d-α-hydroxypropionic acid, occurs in muscle, especially after cessation of blood flow. It increases to a maximum in fatigue of muscle and bears a definite relation to that state. Moreover, through the researches of Fletcher and Hopkins,[7] of Hill,[8] and of Meyerhof[9] it has been found that lactic acid bears an important place in the explanation of muscle contraction. When muscle acts, lactic acid is produced, as it is in the resting muscle; but during contrac-

[1] Discoverer of glycogen and one of the greatest physiologists of all time, 1813–1878; professor in the Collège de France, Paris. Page 21.

[2] Irritation and pressure of other portions of the brain cause hyperglycemia and glycosuria. See page 883.

[3] Diabetes mellitus is a group of conditions due to deficient production of insulin in the pancreas (see page 508).

[4] See papers by Benedict and Nash in the Jour. Biol. Chem., volumes since 1922, and especially Nash, T. P., and Benedict, S. R., Jour. Biol. Chem., vol. 61, p. 423, 1924. See also Cori, G. T. (Institution for Malignant Diseases, Buffalo), Amer. Jour. Physiol., vol. 71, p. 708, 1925.

[5] Chapter XV.

[6] Greek *sarx*, flesh. Paralactic, from the Greek *para*, near to, referring to the similarity to fermentation lactic acid of commerce, which is *d-l*-lactic acid (optically inactive) whereas sarcolactic acid is *d*-lactic acid.

[7] English physiologists publishing in the (British) Journal of Physiology. See résumé of their work in Bayliss (reference page 21 of this book).

[8] Hill, A. V., Foulerton Research Professor of Physiology, University of London. See page 379.

[9] Meyerhof, O. Kaiser Whm. Inst. für Biol., Berlin. The Nobel Prize in Medicine for 1923 was divided between Hill and Meyerhof for researches on muscle.

tion, in case of any injury, in fatigue, or especially in heat-rigor,[1] lactic acid increases in the muscle from an average normal amount of about 0.02 g. per 100 gs. of muscle to 0.2 per cent. or higher.[2] The presence of lactic acid in fatigued muscle may be demonstrated as follows:[3] Flex the head of a frog on its body by holding the animal as one would a pistol, the head placed toward the direction of the outstretched index-finger. By means of a scalpel make a superficial, transverse cut through the skin; then "pith" the frog by passing a probe of stiff wire between the vertebræ beneath the cut and directed backward through the spinal canal, thus destroying the spinal cord. Reverse the direction of the instrument and destroy the brain. Place the body directly upon ice and leave for fifteen minutes or longer. Sever one of the hind legs at the junction of the femur and pelvis and return the body to the ice. Then proceed to stimulate the muscles of the other leg electrically by way of the exposed sciatic nerve. This is accomplished by laying the frog on its back, making a longitudinal incision through the skin from the symphysis pubis[4] toward the head for about 3 cms., parting the muscles along the linea alba,[5] and exposing the lumbar plexus where the sciatic nerve takes its origin. Apply a battery current to this plexus of nerves, regulating the current by means of an inductorium, so that the leg performs flexing movements. Cause the leg to contract for ten minutes or longer, then sever the leg from the body as in the first, or control leg and remove the skin. Repeat the skinning process with the first leg on ice, and then place each leg in a cold mortar containing a small amount of cold sand and ice-cold 95 per cent. ethanol. Grind the contents of each mortar to a pulp and pour this and a small amount of alcohol into a beaker. Repeat with the second mortar, pouring the contents into another beaker. Place the beakers in water at about 50° C. for a short time, then filter. Evaporate the filtrate to dryness below 80° C.,[6] then take up the residue with a small amount of distilled water, aiding solution with a rubber-tipped stirring-rod ("policeman"). Filter

[1] By rigor is meant a rigid condition of the muscle; immediately after death rigor mortis, or death-stiffening, occurs.

[2] Foster, D. L., and Moyle, D. M., Biochem. Jour., vol. 15, p. 334, 1921.

[3] Through the co-operation of the physiological laboratory and the biochemical department it is possible to offer this demonstration as a class exercise, and its importance warrants the interest of both departments.

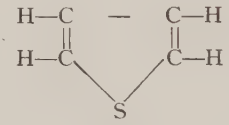
[4] The junction in the midline of the two bones known as the pubes, the front bones of the pelvis.

[5] Latin *linea*, line, and *alba*, white. The central tendon of the rectus abdominalis muscles.

[6] Boiling-point of lactic acid 83° C.

and again evaporate the filtrate to dryness. To the residue, which contains lactic acid, add 3 mls. concentrated sulphuric acid, and with 2 mls. more wash the solution into a test-tube. To the contents of the tube add 3 drops of a 50 per cent. cupric sulphate solution and mix by rotating the tube between the palms of the hands. Leave the tube in boiling water-bath for five minutes; then cool under the cold water-tap and add a few drops of Hopkins' lactic acid reagent,[1] agitating the contents of the tube as additions are made. Replace the tube in the boiling water-bath and note the differences in color in the two tubes, (1) control and (2) fatigued.[2]

Exercise 8.—*The Hopkins Reaction for Lactic Acid.*—The procedure has just been given. The reddish color is produced by aldehyde condensation produced by the action of sulphuric acid on lactic acid and the reagent thiophen,



Thiophen.

The reaction is similar to the Selivanoff test for ketoses.[3] The test is satisfactory only when the lactic acid is present in concentrated form. This is done by extracting with ether and concentrating; the test is performed on the residue.

Exercise 9.—*Uffelmann's Test.*[4]—Place 5 mls. of the reagent[5] in a test-tube and add, by layering, the suspected fluid. At the line of junction of the two fluids a yellow zone appears if the test is positive. Agitate the contents and note that the color permeates throughout the fluid. The limit of sensitiveness of the test is about that of the lactic acid content of fatigued muscle (0.2 to 0.4 per cent.).

The reaction is used largely in the study of stomach contents, the presence of lactic acid indicating fermentation. The reaction is given by all acids, whether organic, like lactic, or mineral, like HCl, and the color produced varies with the hydrogen-ion concentration; in a strong solution ($p\mathrm{H} \leqq 2$) the reagent is decolorized. The color

[1] Appendix.

[2] The instructor may make this Exercise a quantitative one by using a good method, like that described by MacLeod, J. J. R., Jour. Lab. and Clin. Med., vol. 7, p. 1, 1922.

[3] Page 170.

[4] Page 450. Uffelmann, Jules, German physician, died 1894.

[5] Appendix.

changes are due to the ionization of iron derived from the ferric chlorid of the reagent.

Lactic Acid and Muscle Contraction.[1]—Lactic acid, as we have stated, is optically active; it is dextrorotatory. Lactic acid plays an important part in muscle contraction. We have shown that the metabolism of glucids is virtually the same as the chemistry of fermentation[2] and that lactic acid[3] may be derived from glucose and, by reversibility, converted into glycogen. Meyerhof found that there are two periods in muscle contraction, (1) a period when muscle glycogen is converted into lactic acid. During this period contraction, accompanied by a small heat production, takes place. The acid is supposed to increase the permeability of the muscle-fibers to water, an experimentally demonstrable fact. (2) A period when lactic acid disappears from the muscle. During this period the membrane becomes impermeable to water and relaxation takes place.[4] At this time there is an oxidation of about one-fifth of the lactic acid, and this gives rise to heat, the heat of combustion, as determined by Hill. The remaining four-fifths of the acid is reconverted into glycogen.[5] It is to be observed that, contrary to older conceptions, the muscle is not a combustion engine, but a chemical machine,[6] wherein the energy of motion (contraction) is not converted heat energy, but is derived directly from chemical energy.

Lactic Acid in the Organism.— Lactic acid plays a rôle in the following processes:

(1) In anoxemia,[7] either from the lack of oxygen or from some disturbance in the oxidative power of the tissues. When phosphorus is fed to an experimental animal, degeneration of the liver occurs and

[1] Page 145. [2] Page 176. [3] Page 174.

[4] This action is similar to the increased turgescence of tissues (so-called "erectile tissues") in which the permeability for fluids is increased. The nipple of the mammary gland and the mucous membrane of the turbinated bones of the nasal cavities are examples. Under vasomotor, or other stimulus, blood or fluids flow into these structures, causing them to become distended. Now in the case of the muscle, distention means contraction, because two ends of the muscle are attached to solid tissue, bone. See Fig. 76, page 145.

[5] See Foster and Moyle (page 377), Biochem. Jour., vol. 15, p. 672, 1921.

[6] Fletcher and Hopkins conceived the muscle to operate by the production of energy from the oxidation of glucose, but Hill has shown that the heat produced is far less than what this theory would demand, and, moreover, that the production of heat takes place after contraction; Meyerhof found that this small amount of heat corresponds to the oxidation of, roughly, one-third of the lactic acid, as explained above.

[7] Greek, *an*, privitive, *ox*, oxygen, and *'aima*, blood; that is, lack of oxygen in the blood.

oxidation is interfered with; lactic acid appears not only in the liver but also in the urine. "Waxy degeneration" of the muscles is accompanied by the increase in lactic acid in these tissues. Lactic acid occurs in tumors, cancers,[1] and during the resolution of pulmonary exudates in pneumonia following the crisis. Wherever pus accumulates lactic acid is found, and in the so-called "fatty-degeneration" it occurs.

(2) In autolysis, or self-digestion of the tissues. Some of these conditions are included in the statements made in (1) above, for probably the resolution of the pulmonary exudate is of this nature. Perhaps in liver atrophy following phosphorus administration autolysis occurs. Glycogen and glucose, representing glucids and glycerol in the fats, yield lactic acid.

(3) An intermediate substance in glucid metabolism. We have mentioned the participation of lactic acid in the metabolism of these compounds.[2]

(4) Infection. Lactic acid is a deterrent to the action of leucocytes, inhibiting phagocytic action. Lactic acid lowers the resistance of the body to infection.

The Water Content of Muscle.—Great and sudden changes in the size of muscles occur which are attributed to changes in water content. Involution of the uterus following child-birth is principally a matter of loss of muscle water. Loss of muscle tone from disuse is accompanied by lowered water content of the muscle. There is an inverse proportion between the water and fat content of muscle. In weight reduction, as in obesity,[3] muscles lose fat, and water temporarily replaces the fat to maintain the original volume.[4]

"Light" and "Dark" Meat.—This is a matter of the functioning of the muscle. In the fowl, which is commonly used for food, locomo-

[1] But in liver diseases of non-cancerous sort lactic acid occurs in as large if not larger amounts than in cancerous.

[2] Page 146. Recent work has shown that insulin does not affect the quantity of lactic acid in blood and tissues, and this would tend to indicate that lactic acid plays no major rôle in the metabolism of glucids.

[3] Latin, *ob*, out. and *edo*, to eat, that is, eaten out; eaten all that one can. Overfatty.

[4] It is a general rule in living things that change in body proportions is inhibited as much as possible. When an angleworm is cut into halves, the first process is that of restoring the proportion of parts, and later the size is produced equivalent to the whole worm before being severed. The flabby skin of the person who has been obese and who has responded to some treatment (dietary; use of thyroid, etc.) is due to loss of subcutaneous fat, muscle fat being retained longer. Ultimately the muscle water replacing the fat disappears and reduction in volume takes place.

tion by wing is of minor importance, the legs being the principal means of propulsion. Dark muscle is found upon the legs and white upon the breast and wings. In active muscle (dark) there is increased oxidation, more active blood-supply, greater production of extractives, like the purins, creatin, etc. These factors enter into questions concerning the utilization of light and dark meat in diets for invalids. Since there is a larger content of purins in dark muscle, the feeding of such meat to a nephritic, or, to a greater extent, to a subject suffering with gout, is contraindicated,[1] because, as Folin has shown, purins are absorbed by the gouty kidney and produce lesions; and the same results obtain in nephritis.

The Circulatory System.—The vascular system develops very early in the embryo. Blood-islands appear as groups of round cells closely applied to the endoderm, whence they obtain nourishment. Then a differentiation among the cells of the islands appears, wherein the cells lying on the outside or periphery of the island become flattened and surround the rounded cells within. The flattened cells become the endothelium, or wall of the blood-vessel, while the cells lying within form the liquid tissue, blood. These cells are the future corpuscles, white and red. At first both possess a nucleus, but later the erythrocytes cease to multiply and their nuclei leave the cell. The white blood-corpuscle, or leucocyte, retains its typical character of ameboid shape. Hemoglobin begins to form in the erythrocyte before it ceases to multiply and before the nucleus has been extruded. The hematopoietic[2] organs are confined to the marrow of the bones in the normal adult, but in the embryo these include the liver and possibly other organs.

The Leucocytes.[3]—There is much chemical differentiation between the various kinds of white blood-corpuscles, but little is known intimately of their chemistry. Some are capable of being stained with a basic dye (basophiles[4]), some with acid dye (acidophiles), while some are not affected by either (neutrophiles). Phagocytosis is confined to two groups of leucocytes, (1) the macrophage[5] and (2) the microphage, the latter having characteristic nuclei, frequently "sau-

[1] The term "indicated" is used by physicians to mean that a method is favorable to the case in question, and "contraindicated" implies that the result would be unfavorable.

[2] Page 277.

[3] Greek *leyo*, to shine, that is, white, and *kyto*, cell.

[4] Greek *phileo*, to love. For the different kinds of leucocytes see Dorland's American Illustrated Medical Dictionary, 13th ed., Philadelphia, W. B. Saunders Co., 1925, p. 185.

[5] Greek *machros*, large, and *phago*, eat. Similarly, *michros*, small.

sage-shaped;" the longer the nucleus, the older the cell. There is a group of leucocytes known as eosinophiles, containing granules of hemoglobin derived from disintegrated erythrocytes and from other pigment-bearing cells. They are especially abundant during stages of infection and atrophy.

Erythrocytes,[1] or red blood-corpuscles, contain hemoglobin, the red blood-coloring matter. Hemoglobin has weak acid properties which are increased when it is oxidized to form oxyhemoglobin by about 0.02 *p*H (page 84). For convenience hemoglobin is designated HHBO, and oxyhemoglobin, $HHBO_2$. Chemically, hemoglobin, as we have found (page 307), is a conjugated protid, the histon, globin, being united with the colored radicle hematin. The protid fraction has already been discussed (page 324), and we shall now consider the colored, iron-bearing substance, hematin and its derivatives. Hematin may be separated from hemoglobin by acid hydrolysis. The detection of blood is based upon this principle, the hydrochlorid, hemin, being produced (method of Teichmann[2]).

EXERCISE 10.—*Detection of Blood.*—Place some shreds of cotton or other fabric stained with blood on a microscope slide and cover with cover-glass. Now introduce 1 or 2 drops of glacial acetic acid under the cover-glass, the acid flowing in by capillarity.[3] Warm until bubbles of gas appear. Replace the evaporated acid with another drop and let the slide rest in a cool place until crystallization has taken place. Examine the preparation under the microscope for small, garnet-colored crystals, rhomboidal in shape (Fig. 132, page 395).

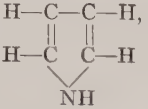
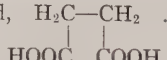
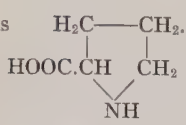
Hemin.[4]—This is the hydrochlorid of hematin. Chemically, it consists of four pyrrol rings,[5] the configuration which was noted in the amino-acids prolin and oxyprolin.[6] The detailed chemistry of

[1] Greek *erythros*, red. Frequently the word is spelled "hæmoglobin," but the Greek source is *'aima*, the *a* aspirated as if *haima*. There is no diphthong, *æ*, therefore in the original.

[2] Teichmann, Ludwig, T. S., died 1895. German histologist.

[3] It may be necessary to add a crystal of NaCl or, better, KI if the stain is old, to afford the halogen.

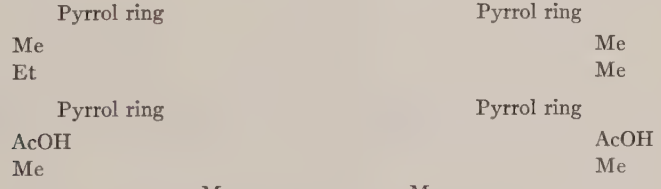
[4] $C_{33}H_{32}O_4N_4FeCl$.

[5] Pyrrol is  , a derivative of succinic acid,  . Prolin is  .

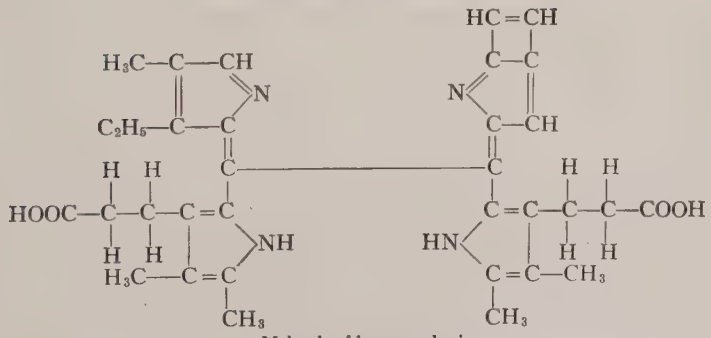
[6] Page 274 and 276.

hematin is not known, but that of derivatives has been analyzed. One of the most significant facts discovered is that chlorophyl, the green coloring-matter of plants, has chemical characteristics similar to those of hemoglobin. Hemin loses its iron and its two carboxyl groups are converted into hydroxyls. *Hematoporphyrin* results, a dicarboxylic acid containing also two hydroxyls; its empirical formula is $C_{33}H_{38}O_6N_4$. On reducing this compound, two molecules of water are lost, and *hemoprophyrin* is formed ($C_{33}H_{36}O_4N_4$). These pigments occur in the urine in certain diseases, such as hepatic involvements and after administration of certain drugs, like di-ethyl compounds used to produce sleep (veronal, etc.). In certain individuals sunlight induces urinary excretion of these pigments. Severe skin reactions result from subcutaneous injection of hematoporphyrin. The similarity in function between chlorophyl and hemoglobin therefore corresponds to the similarity in chemical composition, for chlorophyl is to sensitize the protoplasm of the leaves in their function of manufacturing glucose and starch which are the foods of the plant. Baly, in Liverpool, has imitated both hematoporphyrin and chlorophyl in the use of dyes derived from artificial sources.

The structural formula given by Willstaetter[1] for hemoporphyrin is as follows:[2]



Scheme of molecule of hemoporphyrin.



Molecule of hemoporphyrin.

[1] Willstaetter, R., German organic chemist. See Willstaetter and Stoll, Untersuchungen ueber Chlorophyll, Berlin, J. Springer, 1913.

[2] The abbreviations Me and Et refer to the radicles methyl and ethyl.

The mode of attachment of the iron in the molecule of hemoporphyrin is not known, but it is probably in union with the imid radicle, NH.

Crystals of hemoglobin have been obtained in an earlier Exercise (page 323).

Products of hemoglobin occur in different parts of the body. The pigments of the bile and of the feces are examples.[1] In other cases, however, pigments are not derived from the hemoglobin alone. Thus, urochrome, one of the urinary pigments, has been shown by Roaf[2] to come from chlorophyl of plants. This is readily understood since both hemoglobin and chlorophyl are derivable from a hemoporphyrin-like substance, *etioporphyrin*, a decarboxylated hemoporphyrin. Palmer[3] has shown that other plant pigments, carotein and xanthophyl, allied to chlorophyl, are responsible for certain pigments, such as the *lipochromes* absorbed by fats. These substances, for a time, were thought to be connected with the specific nutritive properties of the fats. The yellow of egg-fat, luteïn, is xanthophyl, a closely allied substance, while butter-fat yellow is carotein. The yellow of human fat is due to two pigments: (1) Carotein and (2) xanthophyl. While fat is the main carrier of these pigments, protid acts in this capacity in the blood. The fact that these plant pigments are refractory to the action of digestive juices accounts for their presence in the human body. The pigment of bile will be considered later, but it may be said that carotein and xanthophyl are soluble in bile; and the former, at least, appears in this fluid, having been isolated from biliary calculi.[4] The plant pigments are excreted in the urine as urochrome.[5]

Hematoidin[6] is similar to if not identical with the bile-pigment, bilirubin, which will be discussed under the heading "bile." Hematoidin is found in old extravasations[7] such as occur after a bruise. It is free from iron. The empirical formula is $C_{16}H_{18}N_2O_3$.

[1] Page 476.

[2] Physiologist to the London Hospital Medical College, London, England. See Biochem. Jour., vol. 15, p. 687, 1921.

[3] Palmer, L. S., contemporary, biochemist to the Agricultural Department, University of Minnesota. See Jour. Biol. Chem., vols. 17 and 39, 1914 and 1919.

[4] See Palmer, L. S. Carotinoids and Related Pigments. New York, Chemical Catalog Co., 1926.

[5] Chapter XV.

[6] Greek *'aima*, blood, and *oidos*, like.

[7] From the Latin *extra*, outside of, and *vas*, a vessel; that is, a blood-vessel.

Hemosiderin[1] differs from hematoidin in several respects: (1) It contains iron; (2) it is quite insoluble; (3) it is retained in the tissues, where it occurs in certain forms of anemia[2] and in other diseases.

Methemoglobin is formed when overdoses are given of certain drugs, such as the "headache medicines," phenacetin[3] and acetanilid, and others like potassium chlorate, permanganates, amyl-nitrite ($C_5H_{11}NO_2$), used in arteriosclerosis, hypertension,[4] etc. Nitrites may form in the alimentay tract in fermentation and give rise to methemoglobinemia; during the process of intestinal decomposition of food residues "enterogenous cyanosis" may arise due to absorption of hydrogen sulphide from the alimentary tract. It is probable that CO_2 of the blood tends to keep down excessive formation of methemoglobin from this source, for the acid CO_2 is able to break up the molecule, giving hemoglobin.

THE SPECTROSCOPIC[5] STUDY OF BLOOD-PIGMENTS

EXERCISE 11.—The fresh blood of a small mammal has been diluted, 1 volume of blood to 30 of distilled water. This serves (1) to hemolyze (page 122) the blood and thus set free hemoglobin; and (2)

Fig. 122.—Direct-vision spectroscope. For interior arrangement see Fig. 123.

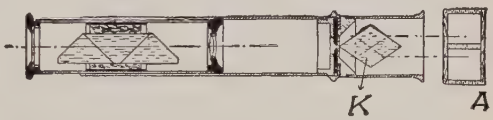


Fig. 123.—Direct vision spectroscope. The eye is applied to the left and the object to the right: *K*, Prism for comparing comparison spectrum with unknown; *A*, knurled head regulating slit.

to dilute the blood, making clearer spectroscopic findings. Obtain 10 mls. of this blood solution and examine it. Do not agitate the solution. Place the tube between the end of the spectroscope containing the vertical slit and the source of light (daylight or artificial light with as continuous a spectrum as possible). A band of colors, red at one end and blue to violet at the other, will be observed through the spectroscope; this is the spectrum. Note that in the

[1] Greek, *'aima*, blood, and *sideros*, iron. Some believe the iron to be an inclusion, and that bilirubin, hematoidin, and hemosiderin are synonyms.

[2] At one time this pigment was supposed to occur in the organs in malaria, but this has been shown to be hematin instead.

[3] Same as acetphenetidin, $C_3H_4.O.CH_3.CH_2.NH.CH_3.CO$; acetanilid (phenylacetamid), $C_6H_5.NH.CH_3.CO$.

[4] By hypertension is meant a heightened blood-pressure such as occurs in old age.

[5] A small direct-vision spectroscope is used (Fig. 122).

region of the yellow, between the yellow and the green, one or two dark bands,[1] running vertically, appear. Remove the tube containing the blood from the end of the spectroscope and note the continuous gradation of colors, without producing any dark bands. Strike a match and hold it carefully in the line of sight through the spectroscope, several inches from the slit; the "D" line, or sodium line is illuminated as a bright yellow band. Now return the tube of blood to the end of the spectroscope and locate the position of the band or bands characteristic of blood. *If there is a single dark band*, the sample contains *hemoglobin*. HHbO. *If there are two bands*, the blood is *oxyhemoglobin*, $HHbO_2$ or CO_2-hemoglobin; these will be distinguished later.

(1) *Hemoglobin.*—If the sample does not show the characteristic single block of dark absorption bands of hemoglobin[2] add a few drops of ammonium sulphide solution,[3] $(NH_4)_2S$, mix, and place in a water-bath not over 50° C. When the color has reached its maximum darkness, examine the contents of the tube by means of the spectroscope; the broad, single band should now be present. Shake the contents of the tube vigorously for one minute and re-examine; oxyhemoglobin has been formed.

Stokes' Solution:[3] This may be used in place of the ammonium sulphide reagent, being more rapid and certain in its action. Heating is unnecessary.

(2) *Oxyhemoglobin.*—Two bands are characteristic of this blood-pigment, the clearness of demarcation and relative size of the blocks being dependent upon the concentration of the blood.

(3) *Carbon Monoxid Hemoglobin.*—The physician is frequently called upon to decide whether death has been due to carbon monoxid, accidental or intentional, as in breathing fumes from a stove or in a closed garage. With the apparatus usually available, that is, a small spectroscope, such as the one used in these Exercises, it is impossible to distinguish between the spectra of oxyhemoglobin and of carbon monoxid hemoglobin. However, according to the following method, this can be accomplished by means of the apparatus mentioned: Place a sample of carbon monoxid blood diluted with water in a test-tube and shake it as before. Examine the spectrum; the spectrum of oxyhemoglobin is given.[4] Now add a few drops of Stokes' solution,

[1] These bands are collections of "Fraunhofer lines," images of the slit, or rather representing the absence of such images, for blood absorbs the colors that would fill the slit.

[2] Figure 124.

[3] Appendix.

[4] Unless some other source of poisoning was employed.

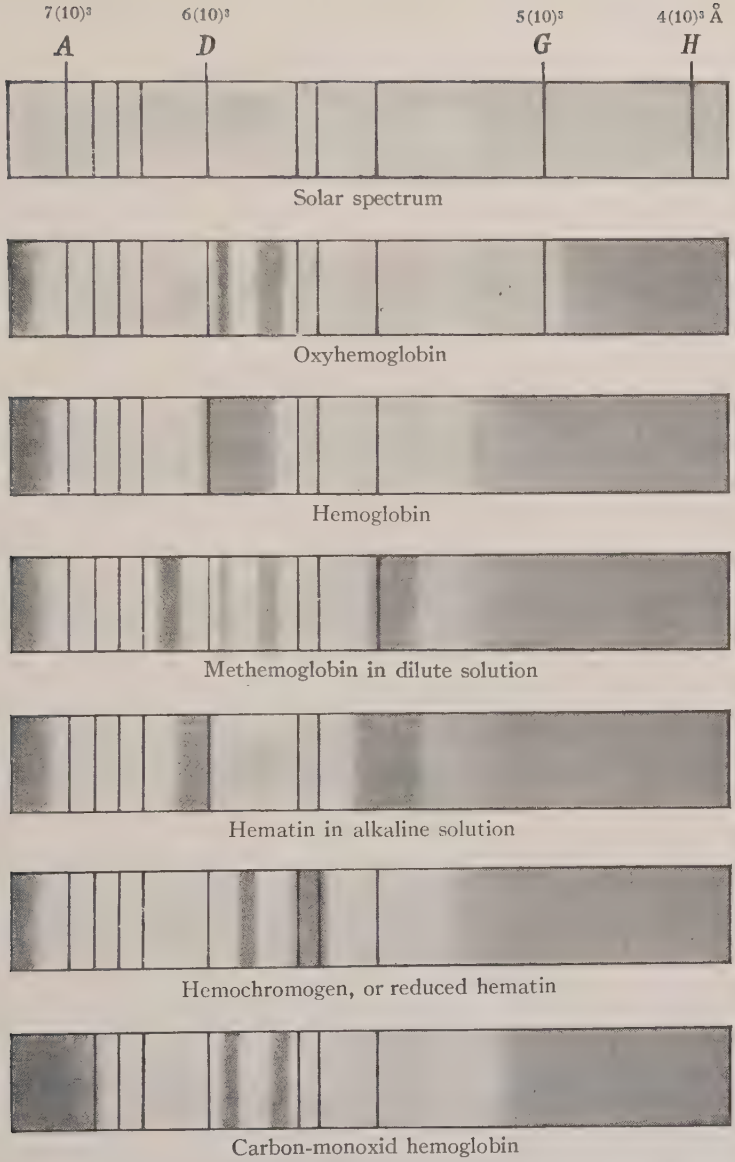


Fig. 124.—Blood spectra. (From Peterson, Haines, and Webster, Legal Medicine and Toxicology.)

mix, and examine the spectrum again. The following conditions hold:

(1) The spectrum is that of oxyhemoglobin; positive for carbon monoxid hemoglobin.

(2) The spectrum is that of hemoglobin; negative test.

The principle of the test is that if carbon monoxid is present, Stokes' solution cannot reduce it to hemoglobin, and it will retain the spectrum similar to that of oxyhemoglobin; if, however, the sample contains no carbon monoxid, the oxyhemoglobin formed on shaking the specimen during the exposure to the air may be reduced to hemoglobin by Stokes' solution.

Apply the test to the samples of blood given you and determine which of the two is carbon monoxid hemoglobin.[1]

(4) *Methemoglobin.*—Prepare the sample of methemoglobin blood as follows: To 10 mls. of the original blood solution used in (1), add

Fig. 125.—Simple hemoglobinometer, Sahli type. The standard is placed in the left-hand tube and the unknown in the right. The unknown is diluted until it matches the color of the standard. The percentage hemoglobin is then read from the scale. Accuracy about 10 per cent.

5 drops of 5 per cent. potassium ferricyanid solution. Mix and note the change in color to a mahogany-brown. Examine with the spectroscope; there is a single band in the red-yellow portion. Prove that methemoglobin may be converted into hemoglobin by Stokes' solution and into oxyhemoglobin by shaking the blood while exposing it to air.

(5) *Urochrome of the Urine.*—Examine a specimen of your own urine with the spectroscope. Note that there are no characteristic bands.[2]

[1] It is well in presenting this Exercise to a class to disguise the odor of the gas used to make CO hemoglobin by the use of a drop of clove oil in each tube.

[2] In concentrated specimens it is observed that the violet end of the spectrum is indistinct.

Methods for Quantitative Determination of Hemoglobin.— The fact that the color of hemoglobin is an index of its concentration permits colorimetric determinations of the content of a given sample of blood to be made. The color is compared with that of a standard solution of hemoglobin or with a substance of similar color.

EXERCISE 12.—*The Tallqvist Method*[1] (Fig. 126).—For clinical work where special apparatus is limited this method may be used for uncritical results. Its limits of error are about 10 per cent. Secure a sheet of the special absorbent paper[2] and have it ready to absorb a drop of blood taken from the finger-tip by the following method:

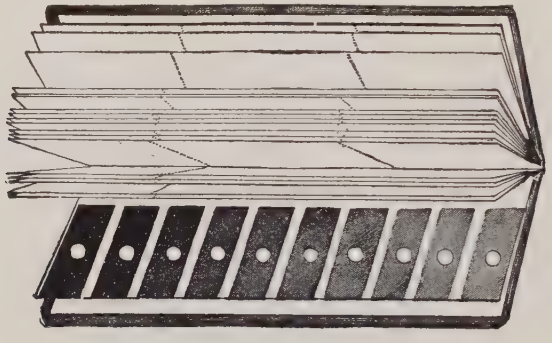


Fig. 126.—The Tallqvist hemoglobinometer. The book contains a chart in colors of different dilutions of hemoglobin, representing blood of health, anemia, etc. The remaining sheets of the book are of absorbing paper designed to receive a drop of blood, which is then compared with the color chart, and the percentage of hemoglobin is read from the matched color.

Break one of the wings from a new pen-point, sterilize it in a flame or in an 80 per cent. ethanol solution and use if for puncturing the skin. Scrub the skin of the finger-tip with a cotton pledget moistened with 80 per cent. ethanol and plunge the point into the fleshy portion of the finger-tip. Discard the first 2 or 3 drops and take the fourth upon the paper. Scrub the wound with the cotton moistened with the alcohol. Let the stain dry of one minute, but no longer, and then compare with the colors in the booklet. These colors are arranged in tenths of per cent. The normal is 80 to 100 per cent. Anemia is

[1] Tallqvist, Theo. W., contemporary, Finnish physician living in Helsingfors, Finland.

[2] Booklets containing sheets of this paper and a color chart used in this method are obtainable from the American concessionary, E. Pennock, 36th and Woodland Avenue, Philadelphia.

indicated with a percentage of about 50 to 70 per cent.; it may run as low as 30 per cent. even in ambulatory patients.

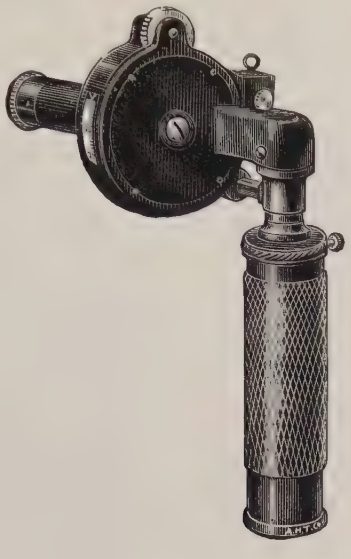


Fig. 127.—The Dare hemoglobinometer. The instrument is held in the hand by means of the handle (right). The eye, applied to the telescope (left), sees the colored scale (the indicator of which is seen on the cylinder to the left) and also the blood, placed in the holder beneath the ring, above, right.

Fig. 128.—Blood glasses for use with the Dare hemoglobinometer (Fig. 127). The blood is held between the two glass plates and these are inserted into the small holder as shown in Fig. 127.

Exercise 13.- *Quantitative Hemoglobin Determination by the Method of Van Slyke.*—Principle: Haldane[1] has found that for the average normal oxyhemoglobin taken as 100 per cent., there are 18.5 per cent. O_2 in each 100 mls. of blood. Hence by observing the amount of oxygen obtained from hemoglobin, the amount of hemoglobin may be calculated. The oxygen is determined in this method

[1] Haldane, J. S., Oxford University, England, contemporary physiologist, known for his researches on blood and blood gases. For the method of his colleague, Barcroft, see Jour. Physiol. (English), volumes 42 and 45. Since the Van Slyke apparatus is used commonly in laboratories for a variety of purposes, the descriptions here are limited to methods utilizing this apparatus.

by mechanically removing it in a vacuum over mercury. A special apparatus is used which is virtually a mercury pump. This apparatus was used in an earlier chapter for obtaining the carbon dioxid in the blood (page 76). Refer to Fig. 36, page 77. A modification

Fig. 129.—Van Slyke blood pipette.

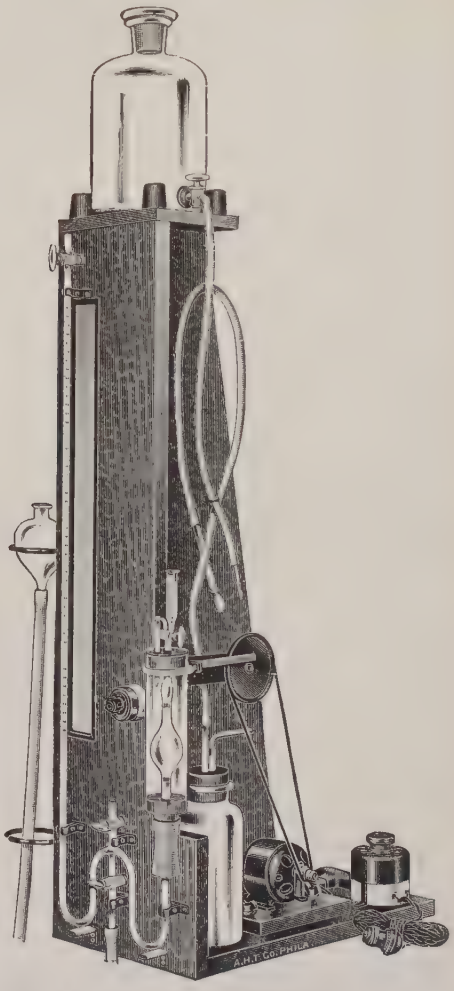


Fig. 130.—Van Slyke gasometric apparatus.

permitting more exact results is shown in Fig. 130. This apparatus will be used later (page 819) for other blood analyses.

Preparation: By means of the mercury leveling bulb attached to *j*, Fig. 36, fill the whole apparatus with mercury and close the two-way cocks. Leave a small amount of mercury in the exit tube *d*; close cocks *b* and *f*, placing their handles transversely. Now intro-

duce into a 500-ml. separatory funnel about 3 mls. of blood[1] and spread it over the interior of the funnel so that the maximal surface of the corpuscles is exposed to the air, thus insuring saturation with oxygen. Five minutes' exposure of the blood to the air is sufficient to insure complete saturation. Stopper the funnel and turn your attention to the mercury apparatus. Introduce into the burette *a* of the apparatus 3 drops of caprylic alcohol[2] to prevent foaming and 6 mls. of 0.4 per cent. ammonium hydroxid solution. Lower the mercury reservoir until the neck is level with the stop-cock *f*, and then cautiously open *b* so that the ammonia solution passes into the

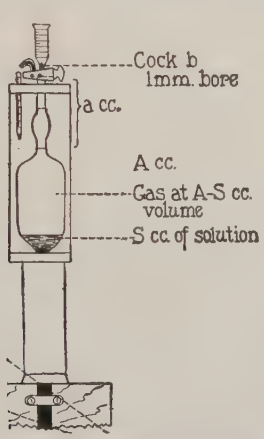


Fig. 131.—Van Slyke's precision vacuum and manometric method for determining blood gases. The method differs from that in which the apparatus ("volumetric type") of Fig. 36, page 77, is used, in that in the present method the volume of a definite size, arbitrarily chosen, is obtained, and the pressure that this volume of gas exerts on a manometer is determined. The apparatus is about the same size as the apparatus figured on page 77. For details, tables, etc., see Jour. Biol. Chem., vol. 61, p. 523, 1924, and for May, 1927, page 120.

graduated tube *c* and thence into the chamber below *c*, admitting no air. Even with the greatest precaution some air enters the apparatus dissolved in the fluids, but this is extracted by the vacuum existing in the apparatus. In order to collect this air close cock *f*, remove the apparatus from the stand[3] with great care and only in the presence of an instructor, and shake the solution around in the chamber for five minutes or longer. Then raise the reservoir above the top of the apparatus and permit the mercury bearing the column of gas and ammonium hydroxid solution to rise in the graduated tube

[1] See page 67 for method of drawing blood.

[2] $C_8H_{16}O_2$. Other substances have been mentioned on page 295.

[3] If the apparatus is mounted on a hinged board, it may be swung without removal.

by opening the cocks *f* and *b*, so that communication is made with the exit tube *d*. Let the columnn of gas pass out through the exit tube, but withold the ammonium hydroxid solution. Close cock *b*, and then lower the reservoir to its former position level with *f*; raise it again immediately and permit about half the column of ammonia solution to pass up into the burette *a*. Introduce by means of an Ostwald-Folin pipette or the Van Slyke mercury pipette (Fig. 129), exactly 2 mls. of the blood well stirred beneath the surface of the ammonia solution in *a*.[1] Then permit the fluid to flow into the graduated tube and into the chamber by proper adjustments of the reservoir and of the stopcocks. Then a drop or two of mercury should be placed in the burette *d* to seal its openings against the introduction of air. Let the apparatus stand for about five minutes. The next step requires great care. Place about 0.5 ml. of saturated potassium ferricyanid[2] solution in the burette and draw it into the apparatus through *b*. Remove the apparatus, or agitate it while on its stand until the blood is spread upon the walls of the chamber for half a minute; then bring the reservoir below the level of the two chambers *c* and *g*, turn *f* so that they are in communication and the mercury and liquid drain into *g*, leaving the gas in *c*. Then close *f* and raise the mercury reservoir above *b*. Open *f* so that *h* is in communication with the chamber and permit the mercury in *c* to rise, carrying with it the column of pure oxygen gas. Adjust the level of the mercury so that the level in *c* is the same as in the reservoir[3] and make the reading. Enter the result in your note-book.

Calculation: You have determined the volume of oxygen at the temperature of the room and at the air pressure that exists at this time and place. To standardize the results convert them into standard temperature and pressure readings (760 mms. Hg and 0° C.) This is done by multiplying the observed reading by 50 to convert to percentage (2 mls. being taken) and by the ratio $(1 - 0.0046t)\ \frac{B}{760}$,[1] B being the barometric reading taken at the time of the determination, and t the degrees C. observed at the same time. Finally, the hemoglobin figure is obtained by dividing the standardized reading by

[1] See Appendix for the method for emptying this form of pipette.

[2] Appendix.

[3] This is to establish the same pressure within the apparatus as that of the air outside; differences in altitude between the observatory and the laboratory must be compensated for. The atmospheric pressure is determined by means of a barometer, or from the Weather Bureau for purposes of calculation.

[4] See Deming, General Chemistry, citation, page 54.

18.5, the ratio of the amount of oxygen in 100 mls. of blood. The following table expedites the calculation (Van Slyke, D. D., Jour. Biol. Chem., vol. 33, p. 127, 1918):

0° C.	Correction for dissolved air. Subtract from burette reading.	Multiply result obtained below by corrected reading. Gives mls. O_2 per cent. blood.	Multiply result obtained below by corrected reading. Gives mgs. $HHbO_2$ per cent. blood.
15	0.037 ml.	$46.5 \frac{B}{760}$	$251 \frac{B}{760}$
16	0.036	46.3[1]	250[1]
17	0.036	46.0	249
18	0.035	45.8	247
19	0.035	45.6	246
20	0.034	45.4	245
21	0.033	45.1	244
22	0.033	44.9	242
23	0.032	44.7	241
24	0.032	44.4	240
25	0.031	44.2	239
26	0.030	44.0	237
27	0.030	43.7	236
28	0.029	43.5	235
29	0.029	43.3	234
30	0.028	43.1	233

[1] These figures are to be multiplied by the fraction given above, $\frac{B}{760}$, in each case throughout the table.

Quantitative methods for carbon-monoxid and for methemoglobin have been devised, both involving the use of the gasometric method just described. The method for methemoglobin uses also the colorimetric comparison of the unknown with a standard. For the theory of the colorimeter see page 711.

Quantitative Method for Determining the CO Hemoglobin Content of Blood.—Method of Van Slyke and Salvesen.—Principle: The method is the same as that given in the previous Exercise in which the oxygen content of the blood is determined, but in the present method both O_2 and CO are removed from the blood, and it is necessary to cause the O_2 to be absorbed. This is accomplished by means of a solution of pyrogallol[1] and alkali.

Procedure: Repeat Exercise 13, using CO-blood up to the point where the reading is taken at the end of the manipulations, when, in place of discarding the gas and cleaning the apparatus for a new determination, the following procedure is followed:

Exercise 14.—Introduce into the burette, *a*, 5 mls. of the pyrogallol solution and about 1 ml. of paraffin oil. Place the reservoir slightly below cock *b*, slowly and carefully turn this cock so as to per-

[1] Page 267.

mit the solution, without the oil, to flow into the graduated tube, and note the shrinking of the gas column due to the absorption of the oxygen by the pyrogallol solution. Now make a reading as before;[1] this reading gives the volume of CO gas.

Calculation: This is done by means of the gas formula: $1.0 - 0.0046t\,\frac{B}{760}$, t being the temperature at the end of the determination and B the barometric reading in millimeters. This multiplied by 50[2] gives the number of mls. of CO held by 100 mls. of whole blood.

Exercise 15.—*Quantitative Determination of Methemoglobin by the Method of Stadie.*[3]—Principle: A distinct red color is imparted to a solution of hemoglobin and of methemoglobin when a cyanid like KCN is added, cyanhemoglobin being formed. The color is proportionate to the concentration of cyanhemoglobin; hence the concentration of cyanhemoglobin may be determined by comparing this color with that of a standard solution of known hemoglobin, or methemoglobin content.

Procedure: Place 2 mls. of blood which has been treated with an anticoagulant[4] to prevent clotting in a 100-ml. volumetric flask and dilute with 50 mls. of distilled water to cause hemolysis, then add 0.5 ml. of a 3 per cent. potassium ferricyanid solution. Let stand fifteen minutes or longer and then add 1 ml. or more of a 0.1 per cent. KCN solution. The color changes to a brilliant orange red due to the formation of cyanhemoglobin. Dilute the solution to the 100 ml. mark, mix well, and compare with the standard solution of ferricyanid-methemoglobin.[5]

Calculation.—This is as follows:

$$\frac{\text{Reading of standard in mm.}}{\text{Reading of unknown in mm.}} = \frac{\text{concentration of unknown (x)}}{\text{concentration of standard.}^{6}}$$

The result is the number of milligrams of hemoglobin plus methemoglobin in the volume of blood taken, namely, 2 mls. Determination of the hemoglobin: This is done upon about 5 mls. of the original methemoglobin blood by the method given on page 389.

Calculation of final result:

Concentration of hemoglobin + methemoglobin	n mgs.
Concentration of hemoglobin	N mgs.
Subtracting, giving methemoglobin	y mgs.

[1] Owing to the very dark color of the pyrogallol solution, a drop or two of water may be permitted to flow down the graduated tube, when a clear meniscus is obtained, suitable for reading. [2] Page 392.

[3] Stadie, W. C. (University of Pennsylvania, Research Medicine). See Jour. Biol. Chem., vol. 41, p. 23, 1920. [4] Page 71. [5] Page 930.

[6] The value of the standard is known by the method given for hemoglobin, p. 389.

The calculations are for 2 mls. of blood; multiply by 50; this gives the final result in terms of milligrams of methemoglobin per 100 mls. of whole blood.

The Detection of Blood.—We have already given one method for this purpose, the hemin test (page 382). This method has been slightly modified by the use of anions other than chlorin:

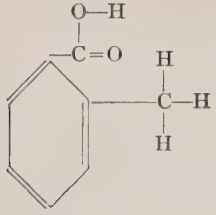
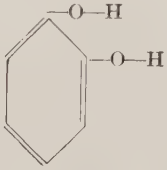
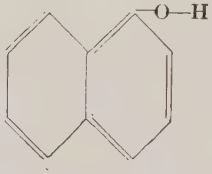
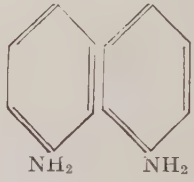
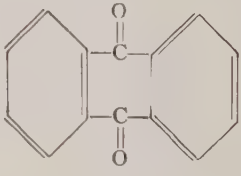
Fig. 132.—Hemin crystals obtained in two tests for blood by Teichmann's method. Note the great difference in size of crystals formed under slightly different conditions (photographs, × 250). (From Todd, Clinical Diagnosis by Laboratory Methods.)

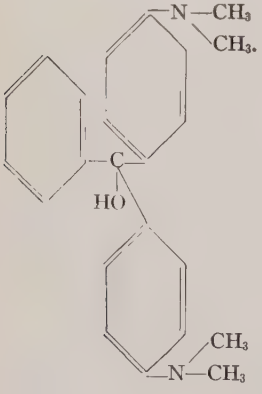
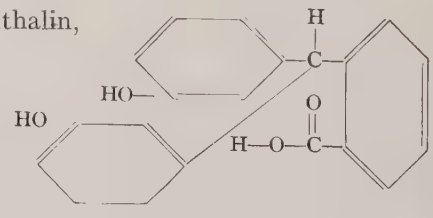
Exercise 16.—Tease apart the cloth fibers upon which there is a blood-stain. Place a small crystal of KCl, one of KI, and one of KBr upon the suspected fibers and cover with cover-glass. Run under one edge of the cover-glass a drop or two of distilled water and heat the slide, gently, with a low Bunsen flame. When the liquid has been evaporated somewhat, add 2 drops of glacial acetic acid as you did in the case of the water, and heat again. Let cool, examine under the microscope for hemin crystals, and compare them with those formed by the Teichmann method (page 382).

Detection of Blood by Methods Depending upon the "Peroxidase" Property of Hemoglobin.—Principle of the Methods.—The hemoglobin decomposes the reagent, hydrogen peroxid:[1]

$$2H_2O_2 \rightleftharpoons 2H_2O + O_2.$$
$$H_2O_2 \rightleftharpoons H_2O + \tfrac{1}{2}O_2.$$

[1] Pages 101 and 118.

The active oxygen then oxidizes certain receptors, like guaiaconic acid,  ortho-hydroxy-phenols such as catechol (pyro-catechin),[1]  and various aromatic substances like α-naphthol,  benzidin,  para-phenylendiamin,[2] anthraquinon,[3] 

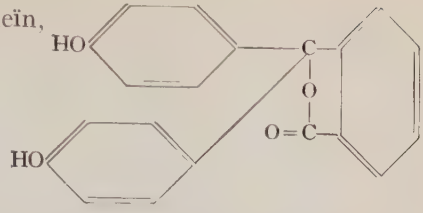
and even as complex a substance as malachite green,  Phenolphthalin, 

[1] Obtained by reduction of guaiacol.

[2] This substance is encountered in toxicology. It is used in commerce in the preparation of furs and often causes a skin rash on persons wearing such furs.

[3] This is the substance which is the basis of the test employing aloes, or aloïn.

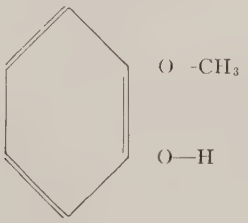
becomes oxidized to phenolphthaleïn,



The following Exercises are designed to give methods for the practical detection of blood and also to show that one is not dependent upon the usual guaiac or benzidin reaction:

Exercise 17.—*van Deen's*[1] *Method for Detecting Blood by Guaiacum.*—Place a dilute solution of blood[2] in a test-tube. Add one-half volume of the special hydrogen peroxid[3] and mix. Then, using a pipette, layer 1 volume of a solution of guaiaconic acid, 1 per cent., upon the solution in the tube. A whitish line of resinous material accumulates at the junction of the two liquids, and immediatey above this a blue color develops, which gradually spreads upward.

The chemistry of the reaction is as follows: Guaiacol,[4]



becomes oxidized by the nascent oxygen, $\frac{1}{2}O_2$, referred to above[5] and guaiaconic acid (page 118) is formed. Guaiaconic acid acts with the iron of the hemoglobin in the presence of chlorin; and a blue color results.

[1] van Deen, Isaac, Holland physician, who died 1869. For reagents see the Appendix.

[2] If the stain is an old one, rub a portion of the stained material in 1 per cent. NaCl solution. Use only a small amount of liquid and keep the solution concentrated.

[3] Made by diluting Merck's "Superoxyl" (30 per cent. H_2O_2) with water to make a 20 per cent. solution. In place of this solution one should obtain a freshly opened bottle of peroxid (3 per cent.) and use 2 volumes of the H_2O_2 to 1 of the solution of blood. The small bottles of H_2O_2 sold at drug-stores and 10-cent stores are preferable to a higher grade of peroxid from a supply that has been exposed to the air and left in a half-filled bottle for some time.

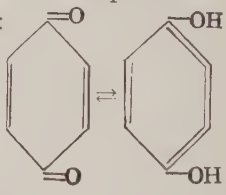
[4] Guaiacol is the mono-methyl ether of pyrocatechin; it is a polyvalent alcohol.

[5] Page 395.

EXERCISE 18.—*Adler's*[1] *Method for the Detection of Blood by Means of Benzidin.*—Place about 2 mls. of glacial acetic acid in a test-tube and warm on a water-bath or cautiously over a low flame. Add as much crystalline benzidin-hydrochlorid as will lie upon the tip of a knife-blade. Mix. Transfer about 0.5 ml.[2] of this solution to a second test-tube and add 10 mls. of 20 per cent. H_2O_2 or equivalent of 3 per cent. peroxid. The appearance of a blue color growing in intensity is positive. Sensitivity: 1 : (10) (page 118). If color appear before the addition of the blood, the preparation must be discarded and the tube thoroughly cleaned with warm cleaning fluid before a new solution is prepared.

Alternative Method.—If several specimens are to be studied within three days, the benzidine may be used in a solution in acetic acid (Appendix) or in ethanol. The procedure is the same as that given above.

The chemistry of the reaction is not thoroughly understood, but it is probable that the *p*-diamino-diphenyl benzidin becomes oxidized to a blue color belonging to the group of "Wurster-red" compounds—substances similar to quinon, quin-hydrone being:



which is green. Wurster-red compounds are partially oxidized *p*-phenylendiamin, and hence the similarity in the use of benzidin and of *p*-phenylendiamin in these blood-tests.

EXERCISE 19.—*Detection of Blood by Means of p-Phenylendiamin.*[3] Place 0.5 ml. of 20 per cent. H_2O_2 or 5 mls. of 3 per cent. peroxid in a test-tube and add 2 drops of 0.5 per cent. aqueous solution of *p*-phenylendiamin-HCl. Mix. Slowly add one volume of the dilute solution of blood. A green color develops. Acetic acid should not be used in this experiment, at least not until after the green color has been obtained; then, if acetic acid is added, the green color changes to a sepia.

[1] Adler, O. and R., Zeitschr. f. Physiol. Chem., vol. 41, p. 59, 1904. Wagner (Arch. f. Verdauungskrankheit, vol. 20, p. 552) modified the test as it is at present.

[2] About 8 drops.

[3] This substance is on the photographic market. As a developer it gives an unusually finely grained negative. The substance is also on the market for the treatment of furs, referred to on page 396.

EXERCISE 20.—*Kastle-Shedd*[1] *Method of Detection of Blood by Means of Phenolphthalin.*[2] Place 2 mls. of the reagent in a test-tube and add 2 drops of 20 per cent. H_2O_2 or 0.5 ml. of 3 per cent. peroxid. Wash this solution into about 100 mls. of distilled water and of this place 10 mls. in a test-tube. Add to the contents of this tube 15 mls. of the blood solution and watch for the development of the usual pink color given by an alkaline solution of phenolphthaleïn. The test is positive with as little as 0.00000004 g. of whole blood.

The Nervous System.—The nervous system is one of the two correlative means of the body, the other being chemical integration.[3] Unless all parts of the body are in intercommunication, disorganization occurs similar to that which takes place when the intercommunicating telephone and telegraph systems are interrupted. Comparable with the telephone, the nervous system has (1) The central offices, the brain and ganglia, and (2) the connecting wires, the nerves. The nerve grows as a pseudopodial outpushing from the cell. Such outpushings may become very long, as, for example, some of the nerve-fibres passing from the sacral region to the foot. The nerves[4] are specialized for the transmission of nerve impulses, while the cells are the trophic (nutritive) portions, containing nuclei. The different cells with their nerves are separate entities, not fused together. The terminals of the nerves are, however, in intimate physical contact, and whatever the nervous energy is, it is enabled to pass from cell to cell through the "dendrites,"[5] or highly branched terminations of the axons. During sleep, it is possible that this physical contact is interrupted by a withdrawal of the tips of the dendrites of one cell from those with which it has been in contact; this withdrawal interrupts the passage of the nerve impulse. Certain drugs, like those of the curare[6] group destroy the "synapse" or junction of the dendrites

[1] Kastle, J. H., deceased, 1916, formerly Professor of Chemistry, University of Kentucky. Well known for his researches on enzymes. Shedd, O. M., Commercial chemist, Lexington, Ky.

[2] Note that this substance is not the ordinary indicator phenolphthaleïn. The formula is given on page 396, together with that of phenolphthalin. The reaction is given also. Phenolphthalin is listed by Coleman and Bell, Norwood, Ohio, as "Phenolphthalin Myers for occult blood." Occult means obscure, that is, blood that is not present in visible amounts. (Latin *ob*, against, and *celo*, sky.)

[3] The hormon system; see page 654. [4] Or *axons.* [5] Greek *dendron*, tree.

[6] This group is not a natural one of chemically similar substances, but many drugs exhibit the power of paralyzing the terminals of the dendritic branches of nerves. Thus, curare, the arrow-poison of the South American Indian; amins, like cholin; guanidin; nicotin; morphin and its allies; strychnin, cocain and its congeners all show the "curare effect." One of the symptoms of the "toxemias of pregnancy" is of this character.

of two adjacent nerve-cells. The nervous system is derived from the ectoderm and hence we find remnants of chemical substances which are typical of the ectoderm, such as neuro-keratin, referred to on page 347. Embryologically, the nerve-cell is, as stated above, an ameboid cell, the pseudopodia of which are the axons. Cells derived from the mesoderm wrap themselves around the nerve-axons and fuse to form a succession of cylinders known as myelin[1] sheaths. A

Fig. 133.—W. J. Gies, Professor of Biochemistry, Columbia University, New York, N. Y. Contributor to the chemistry of the lipids, teeth, etc.

space known as the node of Ranvier[2] lies between two adjacent myelin cylinders. Outside the sheath is the neurolemma, which is derived from the ectoderm and forms an investment for the sheath. Nuclei are easily seen in the neurolemma.[3] At the nodes the neurolemma is contracted upon the axon fiber, the latter being the true nervous part of the "nerve."

The General Chemistry of the Nervous System.—If there is any one chemical entity characteristic of the nervous system it is the lipid con-

[1] Greek *muelos*, narrow, referring to the narrow "tubes," the nerves. The belief was prevalent at one time that the nerves were hollow.

[2] Ranvier, A., French pathologist, died 1922.

[3] Sometimes this sheath is called after its discoverer, the "nucleated sheath of Schwann" (Theodor Schwann, Holland histologist, who, with the botanist, Schleiden enunciated the cell doctrine; died 1882).

tent which makes up 66 per cent. of the solids. The nervous system possesses insignificant amounts of neutral fats, while the lipids such as phospholipids, galactolipids, along with the sterols, make up over 18 per cent. of the wet weight of the brain.[1] We have already studied one of the typical lipids of the brain, lecithin.[2] We shall proceed with the study of the other constituents.

Exercise 21.—Refer to Exercise 7, page 213. Kephalin was saved after removal of lecithin from the brain material. Make appropriate tests for detecting the presence of phosphoric acid, of an unsaturated fatty acid, and of glycerol. After hydrolysis by means of acid, neutralize and make Benedict's qualitative test for reducing sugar.

Neurokeratin forms a framework which remains after the extraction of the lipids. Using some of the shreds of ether-extracted nerve material, make appropriate tests for the detection of glucids.

Galactolipids require special laboratory procedures, and hence they are not studied in these Exercises.

Cerebrospinal Fluid.—The brain and central nervous system do not possess a lymphatic system, such as occurs throughout other portions of the body. The duties of such a system are taken over by the cerebrospinal fluid, which is virtually Locke's solution[3] with some corpuscles, secreted by the choroid plexus. The latter is a tassellated folding of the pia mater coat of the brain, lying innermost of the different membranes and coming into immediate contact with the nervous material proper. This layer is abundantly provided with blood-vessels. The fluid is secreted by the epithelial lining of the villus or tassel which contains small blood-vessels. In the early embryonic condition this layer is simply the roof of the fourth ventricle, the cavity of the hind-brain. As growth proceeds in the mammal the layer is thrown into folds and tucked into the cavity. It is not con-

[1] The water content of the brain averages about 70 per cent., but it varies with age, greater in the young and becoming less up to the age of fifty, then again increasing to the time of death.

[2] Page 213.

[3] Locke's solution is a modified Ringer's solution (Sydney Ringer, English physiologist, died 1910), which was made to resemble the salt solution of the blood. Ringer's solution consists of various salts (see page 138), and Locke, experimenting with the heart, varied the content by adding 0.1 per cent. glucose. The original fluid of Ringer was further modified by Tyrode (French physiologist), who added magnesium chlorid and bicarbonate. Macallum (portrait, page 578) suggests that, since the osmotic pressure and relation to sodium of these solutions is about the same as that of sea-water, blood plasma may at one time in the history of the race have been sea-water incorporated into the body to serve as a circulatory system.

fined to the fourth ventricle, but grows forward through the aqueduct of Sylvius, the third ventricle (of the midbrain), the foramina of Monro, and into each of the two lateral ventricles, I and II, of the cerebrum. The fluid is secreted constantly and passes from the ventricles backward, that is, toward the spinal cord, to the region of outgrowth of the plexus in the roof of the fourth ventricle, where it

Fig. 134.—Technic of spinal puncture. The patient sits on the edge of a chair and bends forward; the crests of the ilia are indicated by black lines, and are on a level with the spinous process of the fourth lumbar vertebra; the "soft spot" is found just above. (From Kolmer, Infection, Immunity, and Biologic Therapy.)

escapes into the subarachnoid[1] space by way of the foramen of Magendie.[2] There is a definite pressure, averaging about 100 mms. of water which is lower than the average blood-pressure (100 mms. of mercury), but higher than that of the blood in the superior longitudinal sinus. The nature and origin of this pressure is still in doubt. The fluid is lost in the sinuses of the dura, probably by a process similar

[1] The arachnoid membrane, lying between the pia mater and the dura, or inner periosteum. The subarachnoid space is that which lies between the pia and the arachnoid membrane.

[2] Magendie, F., died 1855, Professor at the Collège de France, Paris, predecessor of Claude Bernard (page 376) and discoverer of the cerebrospinal fluid.

to that of the secretion of the urine in the kidney. Chemically, the cerebrospinal fluid is normally a comparatively cell-free liquid[1] of low total solids (specific gravity 1.004–6 compared to that of the blood, lymph, 1.055; 1.035; urine, 1.016). The saline content consists mainly of chlorions and the cations sodium and potassium with their molecules,[2] NaCl and KCl, occurring in the ratio 17.3 : 1.0.[2] Cerebrospinal fluid[3] has a higher alkalinity than blood (*p*H 7.35), the average being 7.75; figures as high as *p*H 8 have been reported, but have been shown by Taschiro (Cincinnati) and Levinson (Chicago) to be due to faulty technique. Levinson[4] has shown that spinal fluids on standing unstoppered save for a plug of cotton, lose CO_2 and become more alkaline in reaction. While the fluids of normal and pathological cases show little initial difference in chemical reaction, a characteristic difference develops after they stand. In tubercular meningitis there is more rapid fall of *p*H due to more rapid loss of carbon dioxid. In epidemic meningitis the fluid undergoes autolysis and lactic acid is produced; this leads to an increase in acidity.

The protids of the spinal fluid vary with the location from which the specimen is taken. In the spinal cord the total protid content of the fluid is greater than it is in the ventricles of the brain. The protids resemble in physical and chemical characteristics those of the blood; they consist of albumin and globulin. In certain diseases, like syphilis of the nervous system, the globulin content increases,[5] and this variation is utilized in diagnosis.

In normal fluids:

	G. per 100 mls. fluid.
Total protid[1]	0.075
Globulin	0.008
Albumin, about	0.050

[1] A method for determining the protid content of cerebrospinal fluid is given by Ling, S. M. (Pekin, China), Journ. Biol. Chem., vol. 69, page 397, 1926. He finds from 0.0188 to 0.0379 g. per cent. protid normally.

The so-called "sugar" of the cerebrospinal fluid is undoubtedly glucose in part. On injecting insulin, both blood-sugar and spinal fluid sugar fall, but the fluid sugar does not fall as low, proportionately, as that of the blood. It is preferable to speak of the "reducing sub-

[1] Compare the aqueous humor of the eye, page 405.

[2] Potassium is typical for the cells, which may account for this ratio, since the cell content is low.

[3] Shohl has shown that the CO_2 tension in cerebrospinal fluid is higher than in the normal blood by 5–10 mms. pressure.

[4] Levinson, A., Jour. Infect. Dis., vol. 21, p. 556, 1917.

[5] Page 225.

stances"[1] than of glucose. The reducing substance in the spinal fluid is less than that in the ventricles. The average total reducing substance of 100 mls. of fluid is normally about 0.0525 g.[2] In certain diseases there are characteristic variations in reducing substances, as shown by the following protocol of observations made upon 43 normal subjects and approximately the same number of diseased patients:

Condition.	Appearance.	Cells, 1 cubic millimeter.	Globulin.	Reduction.	Folin's blood method, mgs. per cent.
Normal (43)	Clear	3	−	+	52.5
Luetic (19)	Clear	14	++	+	61.0
Encephalitis (2)	Clear	71	+	+	71.0
	Clear	12	+	+	83.8
	Clear	1	+	+	91.0
Epidemic meningitis	Cloudy	480	+++	+	
Epidemic after serum	Cloudy	...	+++	+	37.0
Brain tumor	Clear	6	+++	+	110.0
	Clear	3	+++	+	83.0
	Clear	4	+++	+	100.0
	Clear	3	+	+	77.0
	Clear	...	+	+	80.0
Tuberculous meningitis	Clear	154	+++	+	24.1
	Clear	5	+	+	34.0
	Clear	20	+	+	21.0
	Clear	21	+	+	20.0
	Clear	...	+	0	18.0
Cerebral abscess[1]	Clear	22	+	+	63.0

[1] Diagnosed in the dispensary as tubercular meningitis, but autopsy revealed cerebral abscess.

The reducing substance of cerebrospinal fluid varies to a certain extent with that of the blood. The following table gives the nitrogenous composition of the blood-serum and corresponding ones for fluid (amounts per 100 mls. fluid):

	Amino-acids, g.	Urea.	Creatinin.	Uric acid.	N. P. N.	Undetermined N.
Blood (normal)	0.007	0.013	0.0015	0.0034	0.030	0.003
Fluid (normal)	0.002	0.011	0.0009	0.0008	0.017	0.051
Blood in uremia	0.0075	0.214	0.0145	0.0180	0.285	0.051
Fluid in uremia	0.0280	0.218	0.0061	0.0045	0.222	

The calcium content of cerebrospinal fluid is about 53 per cent. lower than serum calcium (page 862). In dogs it averages 5.8 mgs. per 100 mls. cerebrospinal fluid (Cameron), which is practically identical with human fluid. This calcium represents the "diffusible

[1] That is, substances affecting Benedict's and Folin's sugar reagents.

[2] This and the following table are taken from B. L. Crawford (Pathologist, Jefferson Hospital), to whom the author expresses his thanks for courtesies. Crawford B. L. and Cantarow, A. Amer. Journ. Med. Sci., vol. 171, page 859, 1929.

calcium" of blood. In the case of tetany, the serum and cerebrospinal calcium are equal in amount.

The Humors of the Eye.—The aqueous humor, lying in front of the lens, is chemically similar to the cerebrospinal fluid. It has a specific gravity close to that of the fluid. The total solids are about the same in the two cases, qualitatively and quantitatively.

The vitreous humor lies behind the lens. It is of entirely different composition; being very loose and gelatinous, it is virtually a semiliquid tissue. Hyalomucoid, a mucoid belonging to the glucoprotids and characteristic of all such tissues, is present in the vitreous humor.

CHEMICAL COMPOSITION OF THE VITREOUS HUMOR[1]

	Ox eye.	Pig.	Horse.
Specific gravity	1.004	..	1.003
Total nitrogen	21.5	19.9	17.8–35.7
Non-protid nitrogen	15.7	13.6	14.2–32.3
Urea nitrogen	9.9	8.8	8–20
Amino-acid nitrogen	1.8	2.8	2–3
Uric acid	2.8	0.45	1–2
Creatinin	1.1		
Creatin	1.6		
Glucose	39	30	71
NaCl	678	705	656
Total sulphur	4	4.5	2
Total phosphorus	2	3.3	

[1] After Cohen, M., Killian, J. A., and Metzger, N., Proc. Soc. Exp. Biol. and Med., vol. 22, page 445, 1925.

COMPOSITION OF AQUEOUS HUMOR[1] (HORSE)

Water	99.6921 gs. per 100 mls. fluid
Solids (100° C.)	1.0869
Protid, total	.0201
Albumin	.0078
Globulin	.0123
Total nitrogen	.0268
Urea as such	.0280
Amino-acids	.0290
Creatinin	.0020
Lipids, about	.0040
Cholesterol	Unidentified.
Reducing sugar	.0983
Sodium	.2787
Potassium	.0189
Calcium	.0062
Magnesium	.0026
Chlorin	.4371
Inorganic P_2O_5	.0033
" sulphur	.0061

[1] Duke-Elder, S. (St. George's Hospital, London), Biochem. Jour., vol. 21, page 66, 1927.

Substances diffuse readily into the aqueous humor. The fluid of the diabetic is hyperglycemic, similarly to the blood. Sodium chlorid, glucose and other normal substances appear in increased amounts in the eye after injection of large amounts into the blood. Unnatural constituents of the aqueous humor also appear after injection, namely, fluorescein, potassium ferrocyanid, potassium iodid, etc.

SUMMARY FOR THE TISSUES

(1) The tissues are differentiated chemically as well as morphologically.

(2) Characteristic chemical substances occur in the various tissues, and these frequently persist when a given tissue becomes altered in its location in the body, as, for example, when the nervous system is invaginated from the ectoderm.

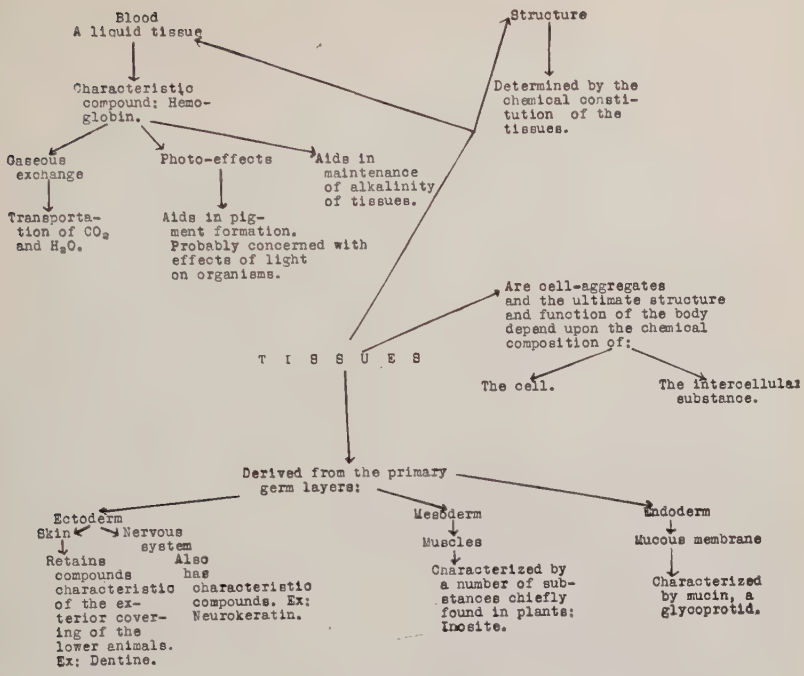
(3) The ectodermal structures are characterized by substances refractory to reagents, giving them their protective properties.

(4) The muscular system is characterized, chemically, by the metabolism of glucids which take part in the functioning of this system; for example, in the metabolism of glycogen and the consequent formation of lactic acid which increases the permeability of the fibers for water, causing them to swell, an essential to contraction phenomena.

(5) The blood is virtually a liquid tissue and possesses certain characteristic chemical substances which aid it in its performance as a carrier of respiratory gases. Cellular changes occur, involving chemical substances, such as the hemoglobin found in the eosinophiles. Methods of detection of blood are based upon the behavior of the iron of hemoglobin, which acts somewhat like an enzyme.

(6) The nervous system is characterized chemically by the content of lipids: Lipids bearing phosphorus (phospholipids), those free from phosphorus (galactolipids), along with the sterol, cholesterol. These compounds are found liberally in the myelin sheath of the nerves. They are all dissolved by the so-called "fat solvents" such as ether. In narcosis, since so many narcotics are fat solvents, it is probable that the essential action is that of entering the myelin sheaths and coming into contact with the nervous tissue proper. However, since all the fat solvents are methyl compounds, their pharmacological function is probably due not alone to their ability to enter the sheath, but also to certain fundamental properties causing a cessation of passage of the nerve impulse.

GRAPHIC SUMMARY


Blood
A liquid tissue
Characteristic compound: Hemoglobin.
Gaseous exchange
Transportation of CO_2 and H_2O.
Photo-effects
Aids in pigment formation. Probably concerned with effects of light on organisms.
Aids in maintenance of alkalinity of tissues.
Structure
Determined by the chemical constitution of the tissues.
Are cell-aggregates and the ultimate structure and function of the body depend upon the chemical composition of:
The cell.
The intercellular substance.
T I S S U E S
Derived from the primary germ layers:
Ectoderm
Skin
Nervous system
Retains compounds characteristic of the exterior covering of the lower animals. Ex: Dentine.
Also has characteristic compounds. Ex: Neurokeratin.
Mesoderm
Muscles
Characterized by a number of substances chiefly found in plants: Inosite.
Endoderm
Mucous membrane
Characterized by mucin, a glycoprotid.

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CHAPTER VIII

THE CHEMISTRY OF COMMON FOODS

"By the mistakes and misconceptions of the past we are guided into more fruitful paths."—*Lafayette B. Mendel.*[1]

In this chapter we shall consider the chemistry of some of the common food substances with a view to acquainting the prospective practioner with the characteristic composition of a few selected foods as a basis for the scientific compounding of diets. Methods for determining the content of protid, glucid, etc., in foods are also presented.

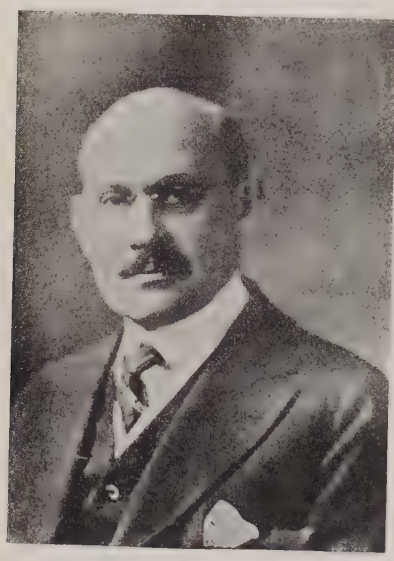

Fig. 135.—Lafayette B. Mendel, Professor of Physiological Chemistry, Yale University, New Haven, Connecticut. Investigator of many phases of biochemistry, but especially, in connection with T. B. Osborne, of nutritional factors.

Definition of a Food.—A food is a substance satisfying in whole or in part the demand of the body for maintenance of form and energy. Accordingly, water, oxygen, cheese, potatoes, etc., are foods. Cheese is capable of temporarily maintaining both the form and the energy of the body; it supplies the demand in part. However, as we shall see later (page 550), the mere supply of the same chemical materials which make up the body in amounts sufficient to maintain energy is not the only consideration. Other substances of unknown composition, designated vitamins, seem to be essential to the proper utilization of food-stuffs. Finally, light of certain wave length seems to be quite necessary for the economy of the body.

Characteristic Composition.—Most foods have a higher content of certain chemical constituents than of others, and are accordingly

[1] Page 24.

classified as "fatty foods," "starchy foods," etc. The content of the three principal organic chemical components is proportioned as follows in bacon, eggs, and bread (in gms. per 100 gms. of substance):

Substance.	Nitrogenous.	Fats.	Starches.
Bacon	9.6	64.0	
Eggs	13.0	10.0	
Bread (white)	7.1	1.2	52.3[1]

[1] This table is after Lusk.

It is evident that bacon is typically a fat substance, while eggs are chiefly nitrogenous, and bread, starchy. Certain foods contribute other substances, such as water, or inorganic salts. Thus milk is 87 per cent. water. It contains only 0.02 per cent. iron as contrasted with 0.2 per cent. in eggs.[1] On the other hand, the calcium is 12 per cent. in milk and only 6.7 per cent. in eggs. A knowledge of the chemical composition, especially of the more common foods, is of the greatest importance to the physician, both in studying the etiology of disease and in prescribing treatment. The following Exercises have been prepared with a view to familiarizing the student with the determination of food content.

Composition of Human Milk.[2]—The content of milk in various organic and inorganic constituents varies through wide ranges. Our knowledge is largely based on German and French studies of wet nurses, but the recent investigations of a group of English investigators[3] on lactating mothers afford more reliable figures. We shall present the subject by giving the characteristic composition of the several constituents and its variation:

Protid.—Average content of total protid for 8 women for 14 days, 2.37 per cent. The highest protid occurs within the first three days. By the end of the first week, the average is 1.4 per cent. Protid values for primiparae[4] are higher than for multiparae.[4] A correlation between protid content and that of ash occurs, a high protid accompanying high ash. The protid rises somewhat towards the end of a feeding.

Sugar.—Average content of lactose, 6.06 per cent. The content varies with the time post partum. The content is low at first

[1] Sherman gives 0.003 g. for 100 gs. of whole egg material.

[2] For a comprehensive account of the composition of human milk see Gardner, J. A. and Fox, F. W. The Practitioner, vol. 114, pages 2, 153 and 680, 1925.

[3] Lowenfield, M. F., Widdows, S. T., Bond, M. and Taylor, E. I. (Royal Free Hospital, London School of Medicine for Women), Biochem. Journ., vol. 21, page 1, 1927.

[4] Mothers with their first child, primiparae; having had more than a single child, multiparae.

(average 5.5 per cent.) and rises to the end of the fortnight. The lowest value found was 4.5 per cent. There is not the clear difference between the lactose content of milk of primiparae and multiparae that is found in protid.

Ash.—For satisfactory ash analyses, 24 hour quantities must be used.[1] Ash behaves in time similarly to protid, high at the beginning of lactation and falling after one week to an average of 0.25 per cent. One-half of the total ash is K_2O, about 1/3.5 of that of cow's milk, the proportion, however, of the various ingredients of total ash being the same in human and cow's milk. During a single feeding, there is slight variation in the inorganic content. *Calcium* is one of the most important constituents of the ash. There is no correlation between the calcium content and the period at which the sample was obtained, as far as the first two weeks are concerned, but later, such a correlation occurs. A variation of from 9–11 mgs. per cent. in the calcium content of human milk from day to day may occur. Thus, three women gave, respectively, averages of 0.0368 per cent. CaO, 0.0573 and 0.0403, for 5 and 6 day periods. The average differences of CaO at the beginning and end of a single feeding were found to be:

With baby	+0.00200
Without	.00800

These variations are negligible. At times it is desirable to differentiate between the functional power of one mammary gland and the other. Calcium, however, cannot be used: The variation in milk content between two mammae was found to be an average of 0.003–0.023 per cent., the latter however, occurring very rarely.

Total Solids.—The total solids vary with the fat content, the range being from 9.6 per cent. to 14.98 per cent.

Exercise 1.—*The Chemistry of Milk.*[2]—Take 25 mls. of skimmed or separator milk in a small beaker and place it in a water-bath at 50° C. Add, drop by drop, acetic acid 1:10, stirring constantly, until a curd appears. Collect the curd and lift it from the whey; see the second part of this Exercise for continuation of the work with the liquid portion, the whey. Place the curd in a large beaker and wash it with distilled water, saving the washings, and adding them to

[1] Holt, L. E., Courtney, A. M. and Fales, H. L., Amer. J. Diseases of Childr., vol. 10, page 229, 1915.

[2] A scheme for practical quantitative analysis of milk is given in Appendix.

the whey. Dry the curd between filter-papers, weigh the mass, and enter the figure in your note-book.

(I) *The curd:* By appropriate tests detect the presence of protid and of phosphorus. Test its solubility in dilute mineral acid (1 per cent. HCl) and dilute alkali (1 per cent. NaOH). Test the solubility of some of the curd in lime-water. What would be the effect of drinking an alkaline solution like bicarbonate before taking a glass of milk? What value would lime-water have in adjusting cow's milk to consumption by the human infant? Knowing that the curd was obtained by bringing the milk to 55° C., what would be the effect upon curd formation of drinking cold milk? Determine quantitatively the content of protid in the curd you have obtained by making a Kjeldahl determination upon a carefully weighed amount (not to exceed 2 gs.) and multiplying the nitrogen figure thus obtained by 6.24. How does this figure compare with the weighed amount of the first of this Exercise? Determine whether fat is present in the curd by shaking a solution of about 5 mls. of curd dissolved in 1 per cent. alkali solution with one volume of ether, letting the mixture settle and then decanting the ether layer into a small beaker (100 mls.), repeating once. Unite the decanted fluids in the beaker and evaporate the ether carefully on the water-bath. Test the residue for (1) fat by the acroleïn test and (2) phosphorus. Is the substance fat? If so, is it a phospholipid?

(II) *The whey:* Transfer 10 mls. of the whey to a test-tube and bring to a boil. Prove that a heat-coaguable protid is present in the whey after removal of the curd. Make tests for inorganic constituents in the whey: Phosphion, chlorion, iron, and calcium. Prove that a glucid is present. Using a protid-free filtrate from 10 mls. of whey, make the osazone test. Can you identify the substance giving the crystals? The heat-coaguable protid consists of a lactalbumin, not identical with serum albumin and, secondly, a lactglobulin identical with the globulin of the blood, known as serum globulin. Lactglobulin therefore is not synthesized in the mammary gland, while lactalbumin is made in that organ.

Exercise 2.—*The Babcock*[1] *Method for Total Fat Content of Milk, Cream, etc.*—(1) Secure two of the Babcock bottles having the narrow stem[2] (Fig. 136), and by means of a special pipette delivering exactly

[1] Babcock, S. M., Professor Emeritus of Agricultural Chemistry, University of Wisconsin, Madison. See the Appendix for the Leffmann-Beam method which was proposed before the Babcock method.

[2] The wider stemmed bottles are for cream (Fig. 137).

Fig. 136.—Narrow form of Babcock milk bottle for determinations of fat in milk. The readings are in grams of fat per 100 gs. of milk. For percentages of fat above 8, use the wider bottle (Fig. 137).

Fig. 137.—The Babcock wide tube milk bottle for estimating the percentage of butter fat in cream, ice-cream, etc.

Fig. 138.—Milk tube suitable for estimating, by the Babcock method, butter fat in human milk, or in 5-ml. amounts of cow's milk. The graduations read in percentages of fat.

Fig. 139.—Apparatus for extracting fat and other substances in biochemical processes. The Soxhlet extraction apparatus, shown in outline, rests upon the water-bath.

17.6 mls.[1] of fluid, pipette from a well-mixed sample of milk, 17.6 mls. of the milk into each of the bottles.[2] Now, holding one bottle in an inclined position, carefully add an equal volume of concentrated sulphuric acid, permitting the acid to flow down the inside of the neck of the bottle and to underlie the milk. At the junction a brownish color will appear, owing to the charring and condensing of the milk by the strong acid. This color, however, must be limited to a narrow zone. Repeat with the second bottle. Then, holding the bottles

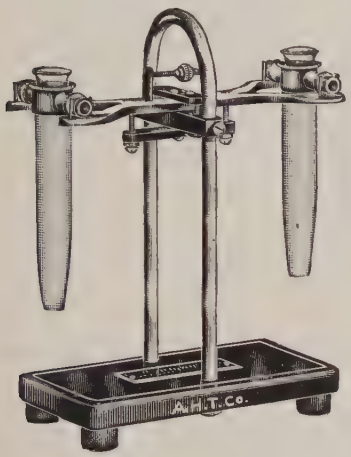

Fig. 140.—Centrifuge tube balance. Centrifuging must be done with the tubes in pairs, one tube balanced against the other.

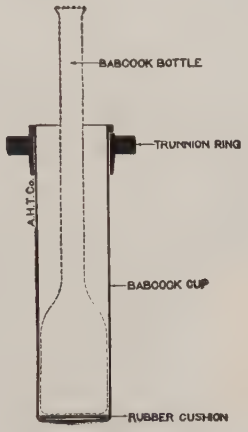

Fig. 141.—Babcock milk bottle in place in the centrifuge cup.

under the cold water-tap, slowly rotate the contents so that they become mixed. The final color should be a light chocolate brown; if the contents of the tube become black, the process must be repeated, using a new preparation. Balance the two tubes on the rough balance near the centrifuge and then place them on opposite sides of the arm or head of the centrifuge, having marked the tubes for identification. Close the lid of the centrifuge box and also the contact. Then run up the rheostat until it is half-way to the fastest speed.[3] Leave about five minutes. In the meantime prepare a flask or beaker

[1] In the absence of these special pipettes, the measurements may be made by means of several measurements of ordinary transfer pipettes of say 5 mls. and 1 ml. (in tenths) capacity.

[2] The purpose of two bottles is that one may be lost in centrifuging and also that one serves as control over the other.

[3] A speed about half the capacity of the ordinary centrifuge (1000 to 1800 R. P. M.); that is, 900 is adequate.

of boiling water and have it ready for use when the centrifuge stops. Remove the flasks one at a time and to each add enough boiling water[1] to bring the layer of fat (recognized by its brown or caramel color) up on the scale on the neck of the flask. After both flasks are removed, return them to the centrifuge and rotate for one minute

Fig. 142.—Type of electric centrifuge in common use in biochemical laboratories. The centrifuge proper is encased in a steel cover to prevent accidents. The electric control is done through the wall rheostat. Usually the speed should not exceed that represented by the movement of the rheostat to middle position. The drawing shows Babcock bottles, 50-ml. and 15-ml. centrifuge tubes. In starting, the rheostat must be on the lowest speed and the increase in strength of current made slowly.

longer. Remove and read the column of fat from the scale.[2] This gives the grams of fat per 100 mls. of whole milk, *i.e.*, the percentage of butter fat.[3]

[1] Or amylol solution: see Appendix.

[2] It is seldom that the column comes exactly on the markings on the scale. One must read, therefore, the column of fat between the ends of the column as they fall upon the scale.

[3] It is important to empty the bottles as soon as the determination is completed and while the contents are warm. Invert the bottle over the sink and shake vigorously to start the flow; rinse with warm water.

(2) *Clinical Method for Determining the Contents of Human Milk.* For the tubes used in this method see Fig. 138. Place exactly 5 mls.[1] of mixed milk in a tube and then pipette as before concentrated H_2SO_4, causing it to layer until the bulb is nearly full. From this point the determination is made according to the description given in (1) above. The readings are, as before, in per cent. of butter fat.

(3) *Exact Method by the Soxhlet*[2] *Extraction Apparatus.*—The apparatus: This consists of three parts: (*a*) a vessel, generally a Florence or Erlenmeyer[3] flask into which is inserted the lower end of the extraction tube (*b*), virtually a large test-tube with two siphon tubes on the sides, to permit the extraction fluid (ether, etc.) boiled from the flask to reach the material undergoing extraction. It also permits this liquid to siphon over into the flask below when it reaches the proper level in the extraction tube. The third part is a condenser (*c*), the least complicated being the Hopkins condenser of the reflux type.[4] Water is passed through this condenser, the right-hand tube being for intake and the left for outlet. Procedure for extraction: Place 150 mls. extraction fluid[5] in the flask and connect this flask with the extraction tube. To prepare the specimen of milk for extraction lay on the balance an alundum extraction thimble.[6] Now tare[7] the thimble against weights, then place a 5-gram weight on the pan of the scale opposite to that holding the thimble. Add milk by means

[1] A mark indicating 5 mls. volume is usually etched upon the tube about half-way up the bulb.

[2] Soxhlet, Frans, German chemist, 1848, Professor Emeritus, University of Munich, Agricultural Department, Munich, Bavaria, Germany.

[3] There are on the market special flasks designed to afford easy and safe connection with the rest of the apparatus. One of the most satisfactory is the Sy flask (Sy, Professor A. P.; head of the Department of Chemistry, University of Buffalo, N. Y.), shown in Fig. 143 modified by Knorr, which has a trough for holding mercury. The end of the extraction tube is slipped beneath the level of the mercury, thus effectually preventing the vapors of ether or other solvent from escaping.

[4] Literally the "flow-back" type; the Liebig type of condenser is usually used for condensing vapors, and the condensed fluid flows down the inclined tube, being caught in a flask and hence not returned to the vaporizing vessel. The reflux type is used in a vertical position, the vapor being condensed and returned to the vaporizing vessel, below, to be revaporized.

[5] Ether is used, as a rule, in milk determinations. It boils at 35° C. Carbon tetrachlorid is non-inflammable, boiling at about the same temperature as ethanol (CCl_4, 77° C.; C_2H_5OH, 78° C.).

[6] Made by the Norton Company, Worcester, Mass., and procurable at chemical supply houses. Alundum is an aluminum composition which is baked hard and withstands great heat, permitting a thorough cleansing of the extraction thimble after use by igniting it in a strong Bunsen flame.

[7] From the Arabic *taraha*, to reject. Balance the paper and the thimble against weights placed on the other pan of the balance.

of a pipette until the instrument is balanced, removing any excess by means of a roll of filter-paper dipped into the thimble. Then transfer the thimble to the electric hot-plate where it is left until it becomes dry. The temperature should be about 50° C. Lower the thimble into the top of the extraction tube. Close the opening by inserting the condenser. Start the flow of water through it and turn on the electricity to heat the solvent in the flask. The solvent vaporizes and the vapors rise through the larger of the two siphon tubes on the extraction tube and enter this tube where they come into contact

Fig. 143.—Modified Soxhlet apparatus for extracting fat, etc., from organic mixtures. The substance undergoing extraction is placed in the compartment *b*. The flask at the bottom is filled with the extracting fluid (ether, for example). The volatile ether rises through the larger tube to the left of *b* and enters *b*. There it is condensed and fills *b* as a liquid up to the level of the upper bend of the siphon tube on the right of *b*; then the liquid siphons into the flask below, carrying with it any extracted substance. A Hopkins condenser completes the apparatus, above.

with the condensing action of the condenser. The liquid thus arising accumulates in the extraction tube and absorbs the fat from the dried milk in the alundum thimble. The condensed vapor rises to the top of the smaller siphon tube, and suddenly the fluid siphons, carrying the dissolved fat into the flask below. After this has continued for some time, generally about two hours, the ether is caught in the extraction tube immediately before it siphons and is removed by disconnecting the flask and the condenser from the extraction tube.

Pour the ether into a vessel. Reconnect the apparatus and collect any remaining ether in the extraction tube. Let the flask cool while stoppered and weigh. Bring the temperature of the flask to 50° C. for one minute, cool, and weigh again. When the weights become constant, enter the amount in your note-book. The figure obtained is the weight of fat per 5 gs. of milk; hence the per cent. of butter fat is obtained by multiplying by 20.

A practical apparatus is that of Dunbar[1] (Fig. 144). The small Hopkins condenser is removed from the large cylinder and the funnel[2]

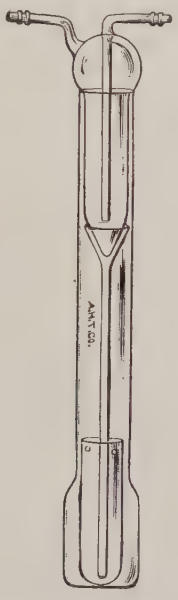

Fig. 144.—The Dunbar extraction apparatus, used in many biochemical procedures, such as determination of lactic acid, etc.

is also withdrawn. The small test-tube resting at the bottom of the cylinder is removed by means of wires attached to the two small openings at the top of the test-tube. A known volume or weight of milk (say 50 gs.) is placed in the test-tube and the water is removed as in the Soxhlet method. Then the cylinder is weighed,[3] the test-tube lowered into the cylinder which rests in a boiling water-bath, or on an electric heater, protected by asbestos. The funnel is lowered

[1] Dunbar, C. O., U. S. Department of Agriculture, Washington, D. C., contemporary.

[2] A pinch of glass wool should be fastened to the lower end of the funnel to prevent too rapid passage of the solvent.

[3] The clean test-tube may be used also as a check.

into the test-tube. Finally, enough ether is placed in the cylinder to rise to the bend in the sides and the condenser is placed in position. Extraction continues for two hours, at the end of which period the ether is distilled into the test-tube. The latter is then removed, emptied, and returned to catch the remainder of the ether. The final traces of ether are removed by placing the cylinder in a boiling water-bath. The cylinder is then cooled and weighed. The results are exactly as in the case of the Soxhlet method.

For methods dealing with the adulteration, preservation, and modification of milk see Appendix.

For the effect of chymosins (the rennins) occurring in the stomach and intestinal juices, which cause curdling and slight hydrolysis, see the chapter on Alimentary Processes, page 453.

Exercise 3.—*Quantitative Determination of Lactose; Method of Folin and W. Denis.*—Principle: This is an adaptation of the method for determination of sugar content devised by Folin and McEllroy,[1] and permits the determination to be made in the presence of inorganic salts and protids. Procedure: Dilute the milk with 3 volumes of distilled water,[2] and place about 12 mls. in an ordinary 50-ml. burette having a well-drawn-out tip, so that drops may be delivered accurately.[3] Adjust the meniscus after raising the end of the tip to make certain that the latter is filled with the diluted milk. Clamp the burette on a burette stand and proceed to the preparation of the copper solution to be reduced: Into a 200 × 20 mm. pyrex test-tube place about 5 gs. of the phosphate mixture[4] and 5 mls. of the special 6 per cent. cupric sulphate solution. Clamp the tube over a micro-burner, or hold it in the hand while permitting a small amount (4 mls.) of the diluted milk to flow into the tube. Bring the contents of the tube to a boil and continue for three to four minutes. Then continue the addition with care and continue boiling, bringing the determination to a close within six to eight minutes. The end-point is reached when the copper is reduced, and cupric thiocyanate, white in color, is formed. Calculation: The cupric sulphate used (5 mls. of a 6 per cent. solution of $CuSO_4.5H_2O$) is exactly reduced by 0.0404 g. of lactose. The n mls. of diluted milk solution necessary to give the appearance of the white color in the copper solution contain exactly

[1] Chapters XV and XVI.

[2] For human milk the dilution may be 1:5.

[3] A special sugar burette is suggested by Folin and McEllroy, Chapter XVI.

[4] Appendix.

0.0404 g. of lactose. One milliliter therefore contains $\frac{1}{n}$ of 0.0404 g. If percentage is desired, this result multiplied by 100 gives the per cent. of lactose in the diluted milk. If the milk were diluted in the proportion 1:4, the per cent. found multiplied by 4 will give the per cent. of lactose per 100 mls. whole milk. Briefly, then:

$$\text{Per cent. lactose} = \frac{4.04 \times \text{dilution factor}}{n}.$$

System for Quantitative Analysis of Human Milk.—In the Appendix will be found a scheme for analysis of small amounts of human milk. The procedures may be employed in analyses of small quantities of milk of animals.

Legal Standards of Milk.—The laws of most states and nations define limits beyond which milk is debarred from sale. In New York, the following stipulations are made: Illegal to sell milk with:

Water.—Less than 11.5 per cent. total solids and more than 88.5 per cent. water; butter more than 16 per cent.

Fat.—Less than 3 per cent.; cream, less than 18 per cent.; butter, less than 80 per cent.

Significance for Clinical Purposes of Variations in Milk Constituents.—Human milk varies from the time it begins to flow until the child is weaned; in fact, it may vary from feeding to feeding. As a rule it grows richer as time progresses, although its total volume per feeding decreases. The solids of milk[1] increase after the third week and continue to do so throughout lactation. Among western peoples lactation seldom continues much longer than one year, but in the East (Japan) lactation may continue two or three years. During this time there is a progressive increase in total solids and in fat content. In the University of Minnesota it has been found that even in the American milk-flow may be stimulated after several days of inability on the part of the mother to nurse the child; also that the time of lactation may be extended apparently indefinitely by keeping the child on the breast. In general, tests of fat content, etc., mean little because the composition of human milk varies in slight degree and a small output of milk at one time is generally compensated for by richer milk later. Lactagogues or galactagogues, that is, substances which increase the flow of milk, as a rule do so by increasing (1) the water content or (2) the fat content of the foods. The drinking of milk or fatty foods, like chocolate, tends to increase the total amount of milk and consequently its total fat content. The milk of domestic

[1] For method of determining total solids in milk see Appendix.

animals, like the cow, goat, ass, etc., differs in composition from human milk. The following table compares the composition of human milk with that of the cow and the goat (from Heineman[1]):

Grams per cent. of substances occurring in milk of man and animals:

Substances.	Human.	Cow.	Goat.
Fat	3.3	3.9	3.8
Lactose	6.5	4.9	4.5
Protid (casein + lactalbumin)	1.5	3.2	3.1
Total salts	0.3	0.9	0.9
Phosphates	0.19	0.58	0.31
Calcium chlorid	0.059	0.119	0.115
Calcium phosphate	0.000	0.175	0.154

Human milk is poorer in fat, richer in lactose, lower in protid, lower in total salts, poorer in phosphates and in chlorids than is the milk of the cow or goat. That of the ass is lower in fat (1 per cent.), but resembles human milk in other respects and is well tolerated by infants. If cow's milk or goat's milk is to be fed to children, it is usually modified by diluting in order to lower the fat content, adding lactose, and sometimes lime-water or $Mg(OH)_2$. This prevents the curd from lumping. In Europe, however, unmodified milk is given to infants taken from the mother's breast. The milk of the Holstein cow is more like human milk than is that of the Jersey. The use of dried milk preparations, etc., will be discussed later.[2]

Exercise 4.—*The Chemistry of the Egg of the Fowl.*—Strike the equator of an egg with a sharp instrument. Into the crack thus formed insert a thumb on each side and carefully open the egg. By transferring the yolk from one half-shell to the other let the white drain into a receptacle. The whitish stringy material adhering to each end of the egg is the chalaza.[3] The chemistry of egg-white resembles that of serum which has already been discussed on page 222. The egg-white will be used in a later exercise in digestion (page 451).

[1] Heineman, P. G., Director of the Cook Laboratories Inc., Chicago, Ill.

[2] Page 554.

[3] Greek, meaning hail-stone, which the chalaza resembles. The formation of the egg in the fowl is as follows: The ovum is produced in the ovary and laid down around it is the nutritive yolk. The egg passes into the oviduct, where it receives a cylinder of egg-white. As it passes down the tube it receives a rotary motion and by this means the ends of the cylinder become closed. This produces the chalaza. Then one membrane is formed around the egg-white and another outside it, leaving a space between the two at the larger end of the egg, the so-called respiratory space. The shell is formed outside this second layer. In the egg of commerce the ovum has generally been fertilized and rests in the blastula stage.

Put the yolk into a pint milk bottle and add 1 volume of ether. Stopper the mouth of the bottle and shake for about two minutes, then decant off the ether, saving it. Add another volume of ether to the residue and shake for five minutes. Decant as before and unite the decanted fluids. Save these and the residue. Add enough 10 per cent. NaCl solution to the residue to dissolve it and then precipitate lecitho-vitellin, the lipo-protid, by (1) filtering the solution on a Buchner funnel, washing the precipitate formed with abundant tap-water; or (2) by dialyzing the solution; or (3) by pouring it in small amounts into a casserole containing tap-water and straining off the precipitate through gauze or filtering it through glass-wool in a large funnel. Save some of the strainings, and to 5 mls. in a test-tube add a drop of glacial acetic acid and boil; note the coagulation of "livetin," a heat-coagulable, phosphorus-poor substance. Now place a small portion of the residue of lecitho-vitellin in a test-tube and add 1 volume of ethanol. Heat the mixture in a boiling water-bath; lecithin goes into solution and the phosphoprotid, vitellin, being insoluble, remains in suspension. Filter, saving the filtrate and residue. Note that the characteristics of vitellin are solubility in reagents and the presence of phosphorus.

Now, returning to the filtrate, add 1 volume of aceton to it and note the precipitate of lecithin. Using the filtrate obtained early in the present Exercise, filter off about 10 mls. of the ether, concentrate on a water-bath in a watch-crystal or small beaker, and examine the residue for crystals of cholesterol.

Eggs as Food.—The fact that the chick develops within the shell into a being capable of leading an independent existence proves that the egg contains all the necessary nutriment. Chemically and functionally, vitellin is to the young chick what caseïn of milk is to the nursling of the mammal. Attention has been directed to the similarity in chemical composition of lecithin and nucleic acid (page 328) and it is probable that the phospholipid is used in the construction of the nucleus. For man eggs make a condensed food, for, whereas the chick requires several weeks to digest the yolk, man requires only two hours. Eggs contain 75 per cent. water, or about the same amount as fat-free steak. However, they contain five times as much lipid material as does beef. The ash content, by weight, is approximately that of beef, but there is a larger amount of phosphorus in eggs. Since the caloric[1] value of a pound of eggs is about 600, and since the average man needs about 3000 Calories, he would require for

[1] Page 596.

maintenance about 5 pounds of eggs[1] in twenty-four hours. If eggs are eaten with plenty of "roughage," that is, indigestible substances which increase the bulk of the feces, there is less danger toward accumulation of gases, such as H_2S, in the digestive tract. The iron content of eggs is conspicuously different from that of milk, as the following table from Sherman shows:

IRON CONTENT OF EGGS AND MILK

	Fresh.	Dried.	Per 3000 calories.
Eggs.....	0.003 g. per cent.	0.0114 g. per cent.	0.057 g.
Milk.....	0.00024 "	0.0017 "	0.010 g.

Eggs are valuable in supplying iron, but this is much better supplied by green vegetables.[2] The phosphorus content of eggs

Fig. 145.—H. C. Sherman, Professor of Food Chemistry, Columbia University, New York, N. Y. Contributor to the chemistry and economics of dietaries, mineral metabolism, etc.

which averages 0.370 g. per 100 gs. dried material (white and yolk) is greater than that of milk (0.215 g. per cent.). Considering the phosphorus content of the yolk alone, it is five times higher than that of milk and twice as high as that of lean steak.[3] It is to be remembered in any discussion of eggs as food that this high content

[1] Equivalent to 45 eggs of the larger kind, like Plymouth Rock eggs. This is equivalent to 5 quarts of milk per twenty-four hours.

[2] Spinach, 0.037 g. iron per 100 gs. dried substance.

[3] The figures given are stated as P_2O_5.

of phosphorus means a liberal contribution of phosphoric acid to the system. It is expressed as 9 mls. normal acid compared with 2 mls. in wheat, 0.1 ml. in corn, -10[1] for potatoes, and -40 for celery. These considerations are of great significance in prescribing diets for nephritics in whom a highly acid urine means low solubility for uric acid.

Eggs contain cholesterol, and in certain diseases, such as cholecystitis and cancer, are contraindicated since cholesterol forms biliary calculi or gall-stones. Experimentally, at least, excessive feeding of cholesterol to the rabbit has been definitely correlated with increased growth of the malignancy present. The paucity of knowledge concerning these matters and their importance should inspire further investigations.

Exercise 5.—*Diabetic Foods.*—Soy-bean meal:[2] Grind 1 g. of beans in a mortar with enough water to form a paste; then stir this paste into a beaker containing about 50 mls. of water. Using 5 mls. of this suspension, perform Benedict's qualitative test for reducing sugar. To another 5-ml. portion add a solution of iodin, drop by drop. Is starch present? To a third 5-ml. portion add about 0.5 volume of filtered saliva, place the preparation in a water-bath at 50° C., and leave five minutes. Perform Benedict's qualitative test. Is a reducing sugar present now? Try Millon's reaction for protids.

Repeat the above tests upon shreds of bran sold on the market for the use of diabetics.

Repeat the above Experiment, using Uneeda biscuit.

Repeat, using toasted cracker or bread.

The Principle of Diabetic Foods.—Soy bean contains no starch capable of producing a blue color with iodin. The bean has a large protid content. There is therefore no immediate contribution to glucose or to glucose-forming substances in the body.

Exercise 6.—*Sugar Content of Candy.*—Dissolve by means of heat a piece of candy, the weight of which has been recorded by the instructor, in 50 mls. of distilled water. Filter off about 10 mls. into a burette and use this liquid to determine by means of Benedict's quantitative method (page 44) the content of the reducing substance. Enter the result of the calculation as the per cent. glucose in the whole

[1] That is, the base-forming properties of potatoes are 10 mls. normal alkali, on the same basis.

[2] The preparations for urease cannot be used because they are adulterated with substances giving reduction.

piece of candy taken. Now, to another 10 mls. of the filtrate add 2 mls. concentrated HCl and leave for ten minutes in a boiling water-bath. Neutralize to litmus, add 1 ml. 10 per cent. NaOH for alkalinity, and repeat the determination for the reducing substance. Enter the result as grams of glucose per 100 gs. of the candy.[1] Compare the figures obtained.

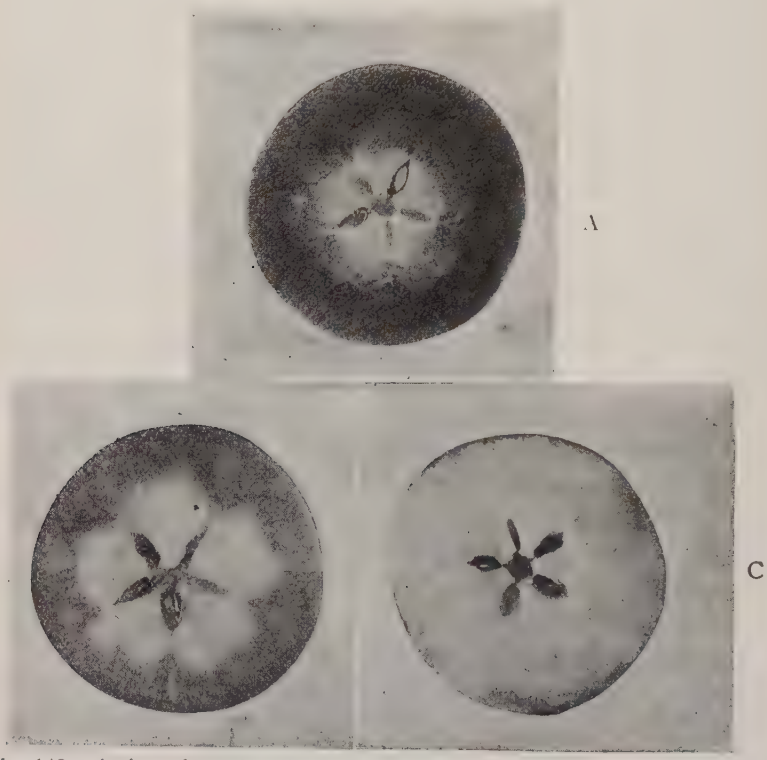

Fig. 146.—Action of amylase in ripening of fruit. A, Apple cut in halves and stained with iodin (black) to show the distribution of starch. B, Apple showing less starch. C, Starch nearly all converted into sugar. For the market fruit is picked in the condition of A and B and left to ripen later. Frequently rotting occurs before the ripening has taken place. (From "Food Products" by H. C. Sherman, revised edition. Copyright 1924 by The Macmillan Company. Reprinted by permission.)

Exercise 7.—*Estimation of Edible Portions.*—Weigh an apple. Peel it, taking care to remove as little of the white meat as possible. Save the peelings and weigh them. Divide the apple into quarters as accurately as possible, saving all parts. Slice one-quarter and place on a pan provided for this purpose. Label with your name, leave in

[1] The weight of the candy is either given you or you will be directed to ascertain its weight.

a drier until the following period. Weigh the residue. From these data compute the percentage of edible portion of the whole apple.

Exercise 8.—Divide one-quarter of the apple used in Exercise 7 at right angles to the axis of the core. Dip one of the cut faces into a beaker containing a small amount of iodin solution (page 147). Leave the apple in contact with the iodin for five minutes. Then wash off the solution with tap-water. Examine the cut face for the amount of starch as indicated by the blue color. Make a drawing in your note-book and compare with Fig. 26 in Sherman, page 342 reproduced in Fig. 146, page 424. Is the apple young, partially ripe, or fully ripened?

Exercise 9.—The butter-fat of ice-cream. Melt a tablespoonful of ice-cream in a beaker, measure, by means of a cylinder, 5 mls. of the liquid and dilute with water to make 18 mls. Place this in a Babcock tube and estimate the amount of butter-fat by the method given on page 411 for milk, using tube shown in Fig. 137.

Exercise 10.—Determine the glucose in 2 mls. of the melted ice-cream by diluting this amount to 10 mls. with distilled water, and making a Benedict quantitative determination for sugar. Express the result in per cent. glucose.

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CHAPTER IX

THE DIGESTION OF FOODS

"Oh, can it be that my life depends upon the smallest vibration of the smallest of those bits of metal!"—*Hugo*, "Notre Dame."

NORMALLY foods find their way into the body of man wholly by way of the mouth.[1] Thence the foods pass through the esophagus to the stomach. Thus far no absorption[2] of the foods from the alimentary tract has occurred. In the stomach the starches capable of being digested are hydrolyzed by means of the salivary enzyme, ptyalin, which accompanies the food into the alkaline portions of the stomach (Fig. 148). Protids are digested by pepsin to the crystalloidal pepton stage in the lower portions of the stomach, where the reaction is acid. Fats are emulsified and to a limited extent hydrolyzed in the stomach by the combined action of bile, regurgitated from the duodenum through the pylorus into the stomach, and the gastric lipase. A certain but unknown amount of fat hydrolysis is due to the H^+ of the gastric juice. Milk is coagulated by the gastric enzyme, rennin or chymosin, and the cheese arising from this process is hydrolyzed along with the other protids. The combined products of gastric digestion, called chyme,[3] pass through the pyloric gate into the intestine, first enter-

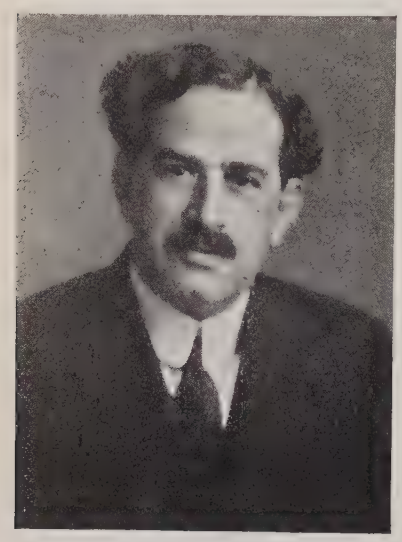


Fig. 147.—C. L. Alsberg, formerly Chief Chemist U. S. Department of Agriculture, now Member of the Food Research Institute, Palo Alto, California. Investigations bearing upon food chemistry, rôle of toxins, etc.

[1] The oxygen, of course, is admitted through the nostrils, but these communicate through the posterior nares with the mouth. In diseased conditions nourishment is sometimes given by rectum, or, in rare instances, by injections into the blood-stream.

[2] Omitting the relatively unimportant absorption from the stomach recently reported.

[3] Greek *chymos*, juice, from *cheo*, to pour out.

ing the duodenum where they encounter the pancreatic juice. Here the starches, still capable of being digested, are hydrolyzed by the amylase, amylopsin; the dextrins arising in salivary digestion are further hydrolyzed to maltase, and the presence of maltase in the duodenal juice insures that the glucid, maltose, arising from the action of ptyalin, is hydrolyzed further to glucose; sucrose is hydrolyzed to glucose and levulose by an invertase, sucrase, and another diglucid-inverting enzyme, acting on lactose (hence called lactase), converts that diglucid into glucose and galactose. Fats are emulsified by the bile and by the OH^- of the intestinal juice, producing some fatty acids and soaps. These soaps aid in the process of emulsification. Most of the fat is hydrolyzed to glycerol and fatty acids by the steapsin[1] of the duodenum. Protids which have been converted into lower aggregates of amino-acids—albumoses and peptones—are hydrolyzed further to peptids and to amino-acids by the trypsin.

After the digestion of the food-stuffs their products are absorbed into the circulatory system. Chapter X will be devoted to this process.

ORAL DIGESTION

The Action of Saliva on Glucids.—The typical action of saliva is to hydrolyze starch to dextrins and maltose. *Ptyalin* is the catalyzer of this reaction. If the enzyme is absent, high temperature and increased pressure are necessary for hydrolysis, unless some other catalyzer, such as hydrion, H^+, is in contact with the starch.[2] In Exercise 2, page 44, the catalytic effect of hydrion is demonstrated. In the following Exercise that of ptyalin is studied:

Exercise 1.—Select a small piece of paraffin wax and chew it in order to increase the flow of saliva.[3] Collect, filter, and use the filtrate for study. Set up the following four test-tubes, each containing 5 mls. of 1 per cent. solution of soluble starch.[4]

To tube (1) Add 1 volume of filtered saliva.

(2) Add 1 volume of filtered saliva and 1 ml. of 0.4 per cent. HCl solution.[5]

[1] Greek *stear*, same as lipon, meaning fat.

[2] Loevenhart, A. S., Professor of Pharmacology, University of Wisconsin, Madison, suggested the term "zymolyte" from the Greek *zyme*, yeast, and *lysis*, to loosen, meaning the material upon which the enzyme works, to replace the awkward expression "substrate." Starch in the above instance would be the zymolyte.

[3] The addition of a small amount of very weak acetic acid will accomplish the increased secretion of saliva.

[4] Page 925.

[5] This approximates the concentration of HCl in the gastric juice.

(3) Add 1 volume of filtered saliva and 1 ml. of 10 per cent. NaOH solution. Determine the hydrion concentration by the colorimetric method (page 71).

(4) Add nothing to this solution; control.

Place these tubes in a water-bath at 50° C. At one-minute intervals remove from each tube separately a few drops of the solution and test on a spot-plate as in Exercise 8, page 149, for the reaction to iodin. When the iodin reaction is given no longer, test 0.5 ml. with Benedict's qualitative solution for reducing substances. Make an osazone test and determine whether maltose or glucose is present five minutes of total time after setting the tubes in the water-bath. The products of salivary digestion average about 20 per cent. of dextrins and 80 per cent. maltose in test-tube experiments; in the body these relations do not hold.

The Oral Enzymes.—Three enzymes have been described as making the composite enzyme "ptyalin:"[1] (1) Amylase, capable of hydrolyzing amylose[2] to the dextrins. (2) Dextrinase, an enzyme capable of hydrolyzing dextrins to maltose and glucose. (3) Maltase, an enzyme capable of hydrolyzing maltose to glucose. Amylase unquestionably is present, as is evident from following the procedure in Exercise 8, page 149. Whether a dextrinase separate from amylase is present has not been satisfactorily demonstrated. Regarding maltase, it is probable that the slight maltose-hydrolyzing action of saliva is due either to bacterial action or to maltase derived from the blood. The fact remains that whether there are three enzymes present or not, glucose, maltose, and dextrins may be obtained from the action of saliva, and existing data indicate that the case is similar to that of pepsin: If pepsin is left in contact with protids long enough, complete digestion products, to and including amino-acids, are produced; if a shorter exposure is made, then intermediate products are formed. Similarly, if starch is left in contact with saliva for a short time, dextrins are formed; if for a longer time, maltose, and finally, a prolonged exposure produces glucose. The products of oral digestion depend upon the time factor.

The Place of Action of Saliva.—Saliva is produced by all the salivary glands, and its action comes into play first in the mouth. However, with the present rapid manner of eating, starchy food is subjected to but slight action of saliva in the mouth. The food is

[1] The word ptyalin is derived from the Greek *ptyalon*, saliva.

[2] Page 150.

swallowed quickly, is frequently washed down with liquid in the form of water or beverages, and consequently remains in the mouth but a limited time. If saliva is to affect the starchy food to any extent it will be in the stomach. The skiagraph of a "normal" resting stomach shown in Fig. 148[1] shows a characteristic position. It is true

Fig. 148.—Roentgen picture of a clinically normal stomach of human adult. Attention is called to the fundus, above, and to the pylorus, preceded by the antrum.

that the shape of the stomach is ever changing, but the salient features of the position of the normal stomach are shown in this picture: The axis of the stomach is obliquely from above downward, inclining from the upper left-hand portion, the fundus,[2] to the lower right-hand portion, the pylorus. The esophagus enters the stomach at the cardia. The starch in contact with the saliva leaves the esophagus

[1] For this illustration the author is indebted to Dr. Willis F. Manges, Clinical Professor and Attending Roentgenologist, Jefferson Medical College and Hospital, and to his colleague, Dr. J. T. Farrel. Likewise, to the subject, A. H. S., the thanks of the author are due.

[2] Latin *fundo*, to pour out, *fundus*, a dish. The description is for the stomach *in situ* in the body.

and is passed to the cardiac portion, where it halts for a brief time,[1] and then is swept by peristalsis[2] into the fundus. The media at this point may be slightly alkaline, acid, or neutral. The stomach is to be thought of as divisible into compartments by movable partitions of tissue, and usually the lower acid portion is more or less definitely set off from the upper alkaline part, but the relations are constantly changing. In the fundus the salivary enzyme or enzymes can exert their effects and hydrolysis of starch takes place. For about half an hour after taking food the fundus remains very slightly acid, neutral, or alkaline. Buffer substances in the food retard free acidity. The movements of the stomach cause a mixture of the gastric juice and all parts become acid. This terminates the action of the oral enzymes.

The Effect of Drinking Fluids during Salivary Digestion.—If water or other liquid containing some salt opaque to the Roentgen rays (*"x-rays"*) is administered to a subject and the process of swallowing is watched by means of the fluoroscope,[3] it is found that the liquid does not enter the fundus proper, but passes directly through the shorter axis of the stomach to the pylorus. Unless solid food or other stimulus causes the pylorus to close, the water is passed immediately into the duodenum. If, however, the pylorus is closed,[4] the water follows the food in its movements through the stomach. Water taken with starchy food does not necessarily dilute the fluids of the fundus, where starch digestion takes place. Moreover, if the fluid in which the glucid is digested is diluted with water in moderate amounts the process of hydrolysis is aided. Increased relative ionization produces a dilution effect which we have described on page 39. Larger amounts of dilution retard digestion; by diluting the digestion solution with twice its volume of water at the same temperature the action is slowed one-half.

The Optimum Reaction for Ptyalin.—This enzyme differs from others in the response it makes not only to the hydrion, but to other ions, even in minute amounts. A certain concentration of electrolytes is necessary for the enzyme to function at all, and one ion affects the reaction of ptyalin to other ions. The optimum hydrion concentration for ptyalin activity varies according to the cation

[1] About six seconds in the so-called "Kronecker-reflex," after the Swiss physiologist H. Kronecker, Director of the Physiological Institute, University of Bern.

[2] Greek *peri*, around, and *stalsis*, squeezing.

[3] A screen impregnated with crystals of calcium fluorid.

[4] The stomach is held by Wheelon and Thomas to be regulated largely by the nerves. See Modern Med., vol. 2, p. 283, 1920. (H. Wheelon, Seattle, Washington; J. E. Thomas, Professor of Physiology, Jefferson Medical College, Philadelphia.)

present, the optimum hydrion concentration being lower in the presence of the cation Na^+ and higher with K^+. It is necessary, therefore, when speaking of the optimum reaction for ptyalin to determine what other ions are present. With Na^+ present in optional amounts (0.02 per cent to 2.0 per cent., expressed as NaCl), the optimal hydrion concentration is slightly on the acid side of neutrality, namely, *p*H 6.7. While the cation has a marked effect, the anion likewise is of importance because the enzyme forms combinations with certain anions, like Cl^-, Br^-, NO_3^-, $SO_4^=$, $OOC.CH_3^-$ and $PO_4^{\equiv}$. Starch is digested more quickly in the presence of Cl^- than of $SO_4^=$ or $PO_4^{\equiv}$. The following table gives the optimal hydrion concentration where certain anions are present:

TABLE (AFTER MICHAELIS) SHOWING THE EFFECT OF CERTAIN ANIONS ON PTYALIN ACTION

Anion.	Optimal *p*H.
NO_3^-	6.90
Cl^-	6.70
$PO_4^{\equiv}$	6.15
$SO_4^=$	6.15
$OOC.CH_3^-$	6.15

If it is true that the reaction of the tissues[1] is slightly acid (*p*H 6.7 to 6.92) as compared to that of the blood (*p*H 7.35), then it may be said that the *reaction of saliva resembles that of the tissues* rather than that of the blood. This is the average reaction of the mouth of a healthy adult.

Is There a Co-enzyme for Ptyalin?—Ptyalin resembles other enzymes in that it consists in the working condition of two portions: A fundamental heat-stable part and a heat-labile portion of unknown composition. Blood carries this unknown substance, and if saliva be inactivated by exposure to a temperature of above 50° C., it may be reactivated by adding a small amount of blood.

Quantitative Determination of Salivary Efficiency.—Cole suggests that the unit of ptyalin should be that amount which converts 10 mls. of a 2 per cent. solution of soluble starch into the products obtained after ten minutes' digestion. Suppose that saliva is collected and its dilution is 0.4, that is, 2 parts of water to 5 of saliva. By the method of Experiment 2 it is found that in 9.5 minutes, 2 mls. of this saliva

[1] The author has shown that the reaction of the tissues (especially liver) is slightly acid. This conclusion has been reached also by Michaelis (see page 97) and recently by Peyton Rous, who employed the method of vital staining; see Jour. Exp. Med., vol. 41, pages 389, 399, 451, 1925.

convert 10 mls. of 2 per cent. starch into products obtained in a control working for ten minutes. Then:

$$\frac{10}{9.5} \times \frac{1}{0.4} = 2.63 \text{ units of enzyme.}$$

Exercise 2.—*Quantitative Method of Cole.* Principle: The activity of ptyalin is measured by determining the time required for the iodin-starch color to change after the addition of saliva. Determination: Making the digest: Pipette 10 mls. of 2 per cent. soluble starch solution into a test-tube. Add 2 mls. of a buffer solution[1] consisting of phosphate—NaOH solution with a *p*H of 6.7. To insure a proper NaCl content add 2 mls. of 0.5 per cent. NaCl solution. Place a beaker within another beaker, making a thermostat. Maintain the temperature within the smaller beaker at 40° C.[2] Place the tube in this bath. In about two minutes, when the contents of the tube will have reached the temperature of the bath, add exactly 2 mls. of the enzyme solution obtained as described on page 427. Note the time. In the meantime place exactly 3 mls. distilled water in each of 6 clean test-tubes and add 1 drop of 0.01 normal iodin solution. The reading: One minute after adding the enzyme to the tube in the water-bath, and at intervals of one minute thereafter, add just 1 drop of the contents of this tube to one of the test-tubes containing iodin. Continue so doing until the color imparted to the contents of the tube last used is violet. Then examine the set of iodin tubes carefully to detect the first change. Determine the time of this change by the location of the tube in the set. Repeat the determination with diluted specimens of saliva until you have obtained a specimen of unknown dilution giving the first change in exactly ten minutes. Calculation: This is given on page 431. Express the results in percentage.[3]

GASTRIC DIGESTION[4]

The Gastric Enzymes.—The following enzymes occur in the stomach:

[1] Page 73. The buffer is used to insure that the hydrion concentration does not change during the course of the determination and thereby introduce an unknown factor.

[2] This procedure is dispensable if the laboratory provides water-baths or ovens maintained at this temperature.

[3] In the above example, where 2.63 units of ptyalin gave the "first change" in ten minutes, since this result is for 2 mls. of saliva, 1 ml. gives 1.36 units and 100 mls. give 100 × 1.36 or 136 units, which is the percentage.

[4] For a detailed account of gastric digestion and accompanying phenomena see Rehfuss, M. E. Diseases of the Stomach. Philadelphia, W. B. Saunders Co., 1927.

Protidoclastic Enzymes:

Pepsin, capable of digesting certain protids, such as albumin, to pepton.

Chymosin (rennin). The function of this enzyme is to coagulate milk. It is still uncertain whether rennin is an enzyme distinct from pepsin.

Urease, deaminizing urea.[1]

Lipidoclastic Enzymes:

Gastric lipase, capable of hydrolyzing neutral fats and phospholipids.

Esterase,[2] hydrolyzing esters of lower fatty acids.

The Rôle of Hydrochloric Acid in Protid Digestion.—The stomach secretes a strong mineral acid, hydrochloric acid, to the average normal amount of 0.2 per cent. This corresponds to a *p*H of 2.8. The optimum reaction for pepsin is somewhat higher, *p*H 1.77, or nearly decinormal, *p*H 1.01. In critical studies of gastric fistulæ in man Carlson[3] reported a range of free[4] hydrochloric acid content of from 0.08 to 0.40 per cent., with an average of 0.20 per cent. HCl. Total acidity[5] was found to be from 0.13 to 0.45 per cent., with an average of 0.25 per cent. As in all other cases of body fluids the reaction as determined by hydrion concentration is the only valuable criterion.

The Origin of Hydrochloric Acid.—This has best been explained by the theory of Maly:[6] In the blood the salts NaH_2PO_4, Na_2HPO_4, $NaHCO_3$, along with CO_2 and $H_2O(H_2CO_3)$ occur as we have said on a previous page;[7] the following equation expresses the theory of Maly:

$$NaH_2PO_4 + NaCl = Na_2HPO_4 + HCl.$$

That is, the acid phosphate which figures in the alkali reserve reacts with the sodium chlorid present in the blood and hydrochloric acid is

[1] Although urease is not a protid-cleaving enzyme, it is placed among these enzymes for convenience.

[2] Willstätter, R., Haurowitz, F., and Memmen, F. Zeitschr. f. physiol. Chem., vol. 140, page 203, 1924. The distinction between steapsin (pancreatic lipase) and gastric lipase is in their stereochemical relations; steapsin cleaves mandelic acid ester yielding levorotatory mandelic acid and gastric lipase the dextrorotatory form.

[3] Carlson, A. J., Professor of Physiology, University of Chicago (contemporary).

[4] See page 445. That is, ionic and that molecular HCl not bound to protid, etc. Total acidity is titratable acidity (see page 88).

[5] For complete discussion see Collip, J. B. See also Carlson, A. J.

[6] Maly, R. L., Austrian chemist, died 1894. Professor in the University of Graz and founder of the great German biochemical journal, Maly's Jahresbericht.

[7] Page 85, where the equilibria and their participation in keeping the blood at given hydrion concentration were discussed.

formed. The phosphate then becomes basic and may act in the maintenance of the reaction of the blood as a phosphate buffer substance. This substance is carried to the tissues by the blood, taking on CO_2 as follows:

$$Na_2HPO_4 + \underset{(CO_2 + H_2O)}{H.HCO_3} = NaH_2PO_4 + NaHCO_3.$$

One of the reasons for believing that this is the way in which the hydrochloric acid is formed is that during the time when there is the greatest secretion of HCl in the stomach the urine becomes alkaline[1] owing to the utilization of the chlorion in the manufacture of HCl in the stomach. The function of the parietal cells found in the mid-gastric mucous membrane is to divert the products of this reaction properly, the acid toward the lumen[2] of the stomach and the salts toward the blood-stream. Exactly where the acid appears as acid is unknown, but it comes to the surface of the gastric mucous membrane and bathes these cells. There is no reason why the acid may not be present in the secreting cells as well. The old question why the stomach does not digest itself is answered in terms of the protective action of the buffers, which, as long as the cell is alive, are properly directed, but when the cell dies are without a directive agent.

The **function of the hydrochloric acid** is shown by the following Exercise:

Exercise 3.—Arrange three test-tubes as follows:
(1) Containing 5 mls. distilled water.
(2) Containing 5 mls. 0.4 per cent. HCl solution.
(3) Containing 5 mls. artificial gastric juice.

Into each tube place a few shreds of fibrin, which have been washed free from blood and preserved in ethanol. Place the tubes in a water-bath at 40° C. Note the time, the appearance of the fibrin at the beginning of the Exercise, after five minutes, and after fifteen minutes. It will be found that the fibrin in Tube 1 has not changed its appearance to any extent; that the fibrin in Tube 2 has become translucent and evidently has absorbed water, while Tube 3 shows the fibrin partially or wholly dissolved, owing to the combined action of the HCl and the enzyme, pepsin, both of which are present in the artificial gastric juice.

[1] This is the so-called period of "alkaline tide," discussed further on page 685.

[2] Latin *lumen*, a streak of light, referring to the light which appears in the cavity of the stomach, or other organ when it is studied in sections.

The Function of Hydrochloric Acid As an Antiseptic Agent in the Stomach.—Many types of bacteria are incapable of existence in an acid medium such as is found in the normal stomach. The acid acts as a deterrent to their activity. When disease affects the stomach its first effect is generally recognized in lowering the concentration of hydrion in the gastric juice. In pernicious anemia[1] the hydrion concentration is lowered and secretion of acid lessened even to the extent of achlorhydria,[2] and gas and other indications of fermentation of food materials appear. This condition is sometimes relieved by the administration of dilute HCl into the stomach directly by means of capsules or by stomach-tube.

The *cause of the absorption of fluid by protid under the influence of acid* is explained by the theory of colloids[3] as a matter of osmotic pressure. Protid being a colloid, does not diffuse, while HCl, H_2O, etc., of gastric juice, being electrolytes, diffuse. The acid forms a protid salt, a protid chlorid, and while the H^+ and the Cl^- may diffuse freely, the protid does not, and hinders the free diffusion of the electrolyte ions; consequently there tends to be a retention of the ions in the protid. An osmotic pressure is set up which is relieved by the inflow of water. By this means the protid swells.

Hydrochloric acid causes a maximal swelling because (1) it is a monovalent acid and there will be the largest number of ions participating in the action, namely, 50 per cent. cations (H^+) and 50 per cent. anions (Cl^-); if a bivalent acid like H_2SO_4 be used, only one-third of the number of ions are anions, for there are H^+, H^+, and $SO_4^=$, and it is the anion which forms the salt. (2) The degree of dissociation of HCl is high.

The Normal Secretion of Hydrochloric Acid.—The human stomach when deprived of food for more than four hours contains on the average about 50 mls. of fluid ("residual fluid" or "residuum"). This is composed of the secretion of the gastric glands and oral fluids, the saliva, and the regurgitated intestinal liquids (intestinal juice and bile). Of this amount (50 mls.), hydrochloric acid makes up a portion varying with different individuals, the average being equivalent

[1] Other conditions affecting the hydrion concentration are discussed below, page 437. Concerning recent studies pertaining to the antiseptic action of gastric juice see Amer. Jour. Med. Sci., vol. 169, p. 373, 1925, in which juice having an acid content below 0.470 is shown to be of no germicidal value.

[2] Greek *a*, privitive, meaning without, and *chlorhydria*, meaning HCl; that is, without hydrochloric acid. Administration of extract of liver seems to stimulate HCl formation and concomitantly, increase of erythrocytes in anemia.

[3] See page 108. See the discussion of Donnan's theory, page 107.

to 0.30 gs. desk reagent concentrated hydrochloric acid,[1] or about 8 drops.[2] The limits of normal amounts of gastric juice vary from 5 mls. to 120 mls. in a resting stomach. There is a continuous secretion of gastric juice varying from 10 mls. to 60 mls. per hour, which continues even after several days of starvation.

Factors Accelerating the Secretion of Gastric Juice.—We shall omit from discussion the nervous factors, for they form a part of physical physiology, but we may say, briefly, that in a state of hunger the sight or thought of food (psychic stimulus[3]) causes a reflex stimulation of gastric secretion. The tenth cranial, or vagus nerve is the path over which these stimuli pass. Hypnotic suggestion has been found as an inciting agent; indeed, the hypnotic state alone without suggestion is thought to be excitatory. Appetite and reflex excitation are acquired, for infants up to a certain age show no such reaction. As to chemical factors, we find that water is a potent stimulus. Extractives from meat likewise stimulate secretion, but not promptly; a latent period exists of about half an hour between the introduction of extractives into the intestine and the extra secretion of gastric juice. However, protidtemns, meat extracts, digested meat, etc., are without effect when introduced into the intestine without HCl. Alcohol, strong solutions of sodium chlorid (10 per cent.), lipidtemns, and other substances excite secretion when introduced into the duodenum. Alkali, like Na_2CO_3, depress secretion. Beverages such as tea and coffee may cause secretion, but the pure alkaloids (caffein, etc.) do not. Pepper and mustard do not affect the secretion. It has been shown that experimentally, at least, bitter substances, such as gentian and strychnin, used in medicine to improve the appetite, do not do so by stimulating the secretion of gastric juice. The presence of solid material, such as unmasticated food and casein clots, does not

[1] That is, the average total acidity of the resting stomach is expressed as 30 mls. of decinormal alkali, and since this is the same volume as 30 mls. of decinormal HCl, we may convert volume to mass by multiplying 30 by the weight of pure HCl in 1 ml. of decinormal solution; since there are 36.4 gs. HCl in a liter of normal solution of HCl, and 3.64 gs. HCl in a decinormal solution, each ml. of the decinormal solution will contain 0.00365 g. dry HCl gas. This multiplied by 30 gives 0.109 g. of dry HCl. The desk reagent is approximately a third concentration of HCl, and multiplying by 3 we have 0.327 g. desk reagent concentrated HCl.

[2] Therapeutically, about 15 drops of dilute HCl are administered in hypoacidity. United States Pharmacopeia IX (9th edition of the U. S. Pharmacopeia) dilute hydrochloric acid is 10 per cent. strength; hence 15 drops are equivalent to 1.5 drops concentrated HCl. Since 8 drops of concentrated HCl occur in the residuum of the stomach, this dose is 18.8 per cent., or less than one-fifth of the normal amount.

[3] The psychic stimulus causes secretion of the juice for thirty minutes. It has been computed to represent about 20 per cent. of the total secretion stimulated by a meal.

increase gastric secretion. This is of interest in the trauma theory of cancer.

The Gastrins.—Certain substances, chemically resembling amins derived from the amino-acids (*e. g.*, histamin from histidin[1]), distinct from them but bearing the iminazol ring,[2] are capable, when injected hypodermically, of exciting the secretion of gastric juice.[3] These substances are known as gastrins. They cannot be caused to act through oral administration, and when injected directly into the blood-stream they are less effective than when injected hypodermically or intramuscularly. Whatever these substances are, they are known only by their experimental manifestations, and Carlson believes them to be of no significance physiologically. Later we shall refer to other substances, known as secretins, also supposedly connected with the inciting of the gastric glands to secretion.

The Location of the Hydrochloric-acid-forming Cells.—This has been studied by Radasch,[4] who finds the relations expressed by the following diagram of the human stomach (Fig. 149). If a line be drawn from the entrance of the esophagus into the cardiac end of the stomach along the lesser curvature to the pylorus, and this be divided into one hundred parts, hydrochloric acid secreting cells are found between the fifth and sixtieth division. Along the greater curvature the glands occupy 78.2 per cent. of the distance from the esophagus to the pylorus; along the dorsal aspect of the stomach they extend 83.5 per cent., and along the ventral surface, 76 per cent. Through the areas thus marked out on the mucosa of the stomach hydrochloric-acid-secreting cells, the so-called oxyntic cells,[5] are distributed fairly evenly. *The fundus of the stomach and the constriction immediately preceding the pyloric vestibule are the acid-secreting regions of the stomach.*[6]

[1] Page 273. [1] Page 272.

[3] Ivy and collaborators find that such substances act similarly when injected hypodermically into an animal in which a subcutaneously transplanted gastric pouch is made. Such a pouch contains acid after an injection of histamin or gastrin subcutaneously anywhere in the body.

He transplanted the tail of the pancreas into the manmary gland and found that 0.1 N. HCl + secretin caused secretion in the transplanted prancreas. Therefore, probably, a harmone incites the gland.

[4] Radasch, H. E., Professor of Histology and Embryology, Jefferson Medical College (contemporary).

[5] Greek *oxys*, sour; that is, acid. Compare "oxy-gen."

[6] It is of interest to note that gastric ulcers occur to the extent of 75 to 80 per cent. within the pyloric end of the stomach, which is the portion free from acid-secreting cells. Moreover, the small area surrounding the esophageal-cardiac end of the stomach is a seat of occasional ulcers.

Methods of Determining Gastric Factors:

(1) **The Collection of Samples.**—Rarely the biochemist is permitted to study gastric juice which is obtained directly from the stomach, due to some lesion which has formed a fistula.[1] The first

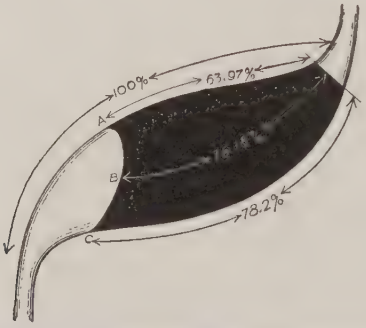


Fig. 149.—Distribution of HCl-producing cells in the stomach. The distance from the cardiac to the pyloric end of the stomach is considered 100 per cent. and the figures give the distances along the different aspects of the stomach which the cells occupy. (After Radasch.) Salivary digestion occurs in the cardiac end of the stomach for about half an hour after ingestion of a meal, although acid cells are located there, because of the buffering action of the foods.

Fig. 150.—Left breast and side (erect position), showing perforation of the walls of the stomach of Alexis St. Martin. (Raymond's Human Physiology.)

study made on human gastric juice derived directly from the stomach is the celebrated case of the French-Canadian, Alexis Saint Martin, studied by the great surgeon and investigator William Beaumont.[2]

[1] Latin, *fistula*, a shepherd's pipe; the term is used because a small tube is placed in the opening through which the fluid is secured from the stomach, first used experimentally by de Graafe in 1677.

[2] The merest sketch of this instance can be given here. For further particulars see Garrison's History of Medicine, Philadelphia, and W. B. Saunders Co., 1921. St. Martin was accidentally shot in the abdomen, and the stomach lacerated so that "a perforation was made directly into the cavity of the stomach through which the food was escaping when first seen." While every effort was made by the surgeon attending

Previous to Beaumont's work gastric digestion was supposed to be a matter of "putrefaction, trituration, fermentation, and maceration." Beaumont's description of the fluid now obtained in a different manner needs no alteration to represent our modern views. "Pure gastric juice, when taken directly out of the stomach of a healthy adult, unmixed with any other fluid, save a portion of the mucus of the stomach with which it is most commonly and perhaps always combined, is a clear, transparent fluid; inodorous; a little saltish and very perceptibly acid. . . It possesses the property of coagulating albumin in an eminent degree; it is powerfully antiseptic, checking the putrefaction of meat."[1]

Experimental fistulæ have definite disadvantages, and in 1890 Pavlov[2] proposed making a "small stomach" out of a portion of the stomach of an experimental animal, an operation known as Pavlov's pouch,[3] which permits the isolation of this sac from the common lumen of the stomach. Gastric juice may be obtained from the opening of the pouch upon the external wall of the abdomen, and food and liquids may be inserted and their fate determined. The most recent important gastric studies are those of Carlson[4] and his colleagues Luckhardt and others, in the Hull Physiological Laboratories of the University of Chicago, where studies were made upon subjects with

St. Martin to close the wound, it never healed, and a permanent fistula remained through which Beaumont obtained gastric juice and noted the effects of foods, etc. This study was made while Beaumont was post surgeon on Mackinac Island, Michigan, in 1822. When it is considered how primitive were the conditions far in the wilderness, no library within hundreds of miles and out of contact with men of similar interests; when it is realized that nearly a century passed before any fundamental addition to the knowledge of gastric action was made to that determined by Beaumont, the magnitude of the work is evident. The picture presented here is offered as a stimulus to the rising generation of physicians to add as they can to medical knowledge wherever they may be and under whatever conditions they may find themselves. For full description of the work of Beaumont see Osler, W., William Beaumont, Jour. Amer. Med. Assoc., vol. 39, p. 1223, 1902. Beaumont published the first description of his work in the Philadelphia Medical Recorder, vol. 8, No. 1, January, 1825 (the year of the founding of Jefferson Medical College); by editorial oversight Beaumont's name did not appear in the article.

[1] Quoted from Osler's article mentioned above, footnote 1.

[2] Ivan Petrovich Pavlov (spelled in Germany and frequently here Pawlow), Director of the Physiological Institute, Leningrad. Professor Pavlov visited the United States in 1923, but, being robbed by a highwayman on his way to New Haven, he returned without participating in the scientific life of the country, a matter of the deepest regret to many American physiologists. Heidenhain, a German physiologist and preceptor of Pavlov, had suggested and used such a method as that usually known by his pupil's name, but Pavlov improved the procedure.

[3] This pouch is figured in Mathews' Physiological Chemistry, 4th ed., p. 355, 1925.

[4] Carlson, A. J. (page 433), and Luckhardt, A. B., Chicago.

esophageal strictures[1] to whom food was administered through an operative gastric fistula, which was never permitted to close.

The *stomach-tube method* is the outgrowth of the earlier use of a catheter first used for feeding and for washing out the stomach, but Leube[2] adapted it to the recovery of his "extract" and for obtaining

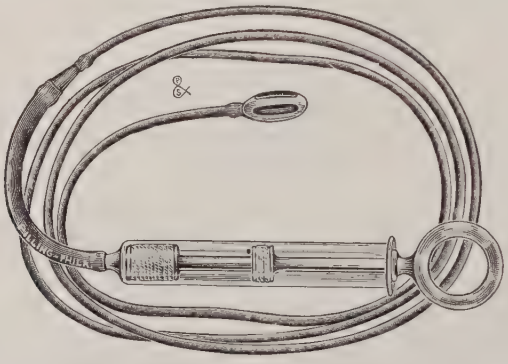


Fig. 151.—The Rehfuss tube for fractional gastric analysis.

gastric secretion in 1867.[3] The small bore tube now in use in gastric studies was introduced by Rehfuss.[4] Lyon[5] has suggested a pear-shaped tip and a terminal as well as slotted holes, with a view to making the withdrawal of the tube more easily done.

Exercise 4.—*Method of Intubation.*—Preparation of the subject: (1) For residual[6] gastric secretion: The day before the test is made the subject is given the following evening meal:

Meat sandwich	1
Raisins	20
Water, glass	1

[1] The walls become hardened and the lumen obliterated, or narrowed, so that food cannot pass. The perfection of methods of treatment by Chevalier Jackson in the bronchoscopic clinic of Jefferson Hospital, Philadelphia, whereby bougies are drawn through the esophagus from a gastric fistula, upward and outward through the mouth, makes possible permanent fistulæ very remote.

[2] Leube, W. D. (pronounced "loyba"), German physician, died 1922. He experimented with predigested meat and invented "Leube's Extract."

[3] Kussmaul, A., German physician, demonstrated the value of the tube in clinical work in 1867.

[4] Rehfuss, M. E., Assistant professor of Medicine, Jefferson Hospital. The tube known as the "Rehfuss tube" was described in the New York Medical Journal, August 22, 1914.

[5] Lyon, B. B. V., Head of the Gastro-intestinal clinic, Jefferson Hospital. See Lyon.

[6] See page 442 for the meaning of the term.

On the following morning the subject does not eat or drink and presents himself for intubation.

Intubation.—Preparation of the tube: The tube is boiled in water for fifteen minutes or longer in order to sterilize it. It is then lifted

Fig. 152.—Students taking the Rehfuss stomach-tube. The student to the left is taking the "olive." The students in the middle of the picture have swallowed the tube and are awaiting the withdrawal of the residuum, as shown to the right, where an instructor is aspirating the residuum from the seated subject. Note the beakers held by the students; saliva is collected in order to prevent admixture with the stomach contents.

out of the water with crucible tongs or forceps and laid upon a folded paper towel which in turn is placed upon chopped ice. The tube is ready for use after the tip has cooled to about 5° C. The subject: Wash out the subject's mouth with tap-water to free the cavity from sputum, food residue, etc. Coil the tube loosely in the left hand and

drop the tip into the fauces,[1] aiding the motion with the tip of the index-finger.[2] Have the subject make swallowing movements and the tip will be carried over the glottis and into the esophagus. Gagging to a greater or less extent frequently occurs, but the use of the tube is free from danger of any kind, and the student who expects to become a successful operator in this technic should master the method of taking the tube himself. Have the subject maintain the tube, after the tip passes into the stomach, back of the molars on one side of the mouth, and keep the free end above the level of the stomach; it is well to fasten the loops of the tube over the ear. A clamp may be placed on the tube before taking the first sample.[3]

Withdrawal of the Residuum.—This may be done either by gravity or by aspirating with a glass syringe. Collect the fluid in a 250-ml. beaker. When no more can be obtained by either method, clamp the free end and remove the syringe.

Measure the residuum and enter the figure in your note-book. Save the fluid.

(2) *For Fractionating Meal.*[4]—With the tube to one side of the fauces slowly eat the modified Ewald meal.[5] Note the time. Avoid swallowing the saliva, which is alkaline and a buffer, and modifies the hydrion concentration of the gastric juice. At intervals of fifteen minutes, for two hours, remove 10 mls. of gastric secretion by syringe and place in a separate test-tube, labeled accordingly. At the last

[1] Latin, *fauces*, plural, meaning the narrowing of the rear of the mouth cavity.

[2] See Fig. 152. This plate was made as a part of the regular exercise in the Sophomore Class course in biochemistry, Jefferson Medical College. In this course nearly every student of the 135 enrolled from year to year takes the tube and performs the various tests outlined in this book.

[3] During this period saliva collects, and the subject should be provided with a small beaker for holding the expectorated material.

[4] Introduced in modern form by Rehfuss after the older work of Schuele in 1895. The older methods consisted in feeding a test meal and withdrawing it entirely after one hour by means of the large stomach-tube. The advantage of the fractionating method is that a much more accurate study of gastric acidity, formation of fermentation products, gastric mobility, etc., can be made than in the static condition of the single withdrawal.

[5] Ewald, K. A., died 1915. German physician, Berlin. The meal consists of toast and tea, the toast being made from the ordinary pound loaf of white bread (2 slices) 15 mms. thick, with the crust and about 1 cm. of the bread beneath the crust cut away, leaving a piece of toast about 8 × 8 cms. square. Liquid in the form of water or, better, tea (350 mls.) is used. The tea may be sweetened with one "domino" of sucrose, or a small amount of lemon or orange juice may be mixed with the liquid. Saccharin may be used for sweetening. Avoid the use of milk or any food containing milk or cream after taking the tea, owing to the formation of an almost indigestible casein tannate.

withdrawal the total contents of the stomach are withdrawn. The examination of the contents from each withdrawal must be made by the student, or his "doctor,"[1] following each aspiration, according to the methods given below. Following the withdrawal of the last portion at the end of the period, drink 225 mls. of tap-water, the average amount of water in a tumbler, and remove this washing by means of the tube; then remove the tube.

(3) *Mobility.*—This may be studied by introducing the following meal about twelve hours previous to intubation:

Meat sandwich	1
Prunes, stewed	6

In the morning the procedure under (1) is employed.

Methods of Analysis of Gastric Contents.—The gastric contents obtained by the above methods are analyzed as follows:

EXERCISE 5.—Tabulate the following factors of the gastric contents obtained in the previous exercise (Exercise 4):

1. Total volume of gastric contents; this is discussed below.
2. Qualitative statement as to relative amounts of:
   - (*a*) Chyme, that is, undigested residue of food: Indicate by + a small amount, ++ for larger amount, etc., comparing your result with that of others of the class.
   - (*b*) Mucus, indicated by +'s as in (*a*): The purpose of this determination is to give advice concerning the emotional state of the person, for in excitement, due to the taking of the tube, etc., this portion is increased and hence the concentration of acid may be diminished.
   - (*c*) Bile: This refers to readily observed bile imparting a greenish tinge to the contents. It does not refer to bile for which a test has to be made. Enter the relative amount of this macroscopic bile as +, ++, etc. The significance is that bile has regurgitated from the duodenum through the pylorus, due to:
     - (1) Normal factors like gagging in intubation, coughing, etc.
     - (2) Pathological factors: Abnormal stimulation of the mucous membrane of the duodenum, or gastric hyperacidity. Operations on the gall tract, ulcer

[1] The class may be divided, one-half being subjects and the remainder serving as doctors making the various examinations.

of the duodenum, etc., are causes,[1] while gastric hyperacidity calls upon the alkaline intestinal secretion as a neutralizing agent, thus causing the regurgitation of the bile along with other intestinal juices.

(*d*) Occult blood: This is detected by the benzidin test given above on page 118. It is indicative of a lesion in the stomach, in the mouth (bleeding teeth, etc.), or of regurgitation from the intestine if a pathological state exists there. Macroscopic blood, that is, red blood, occurring in evident quantities is indicative of an active bleeding in the stomach.

(*e*) Acidity: (1) *Total Titratable Acidity.*[2]—Strain off the suspended matter and filter the strained fluid through

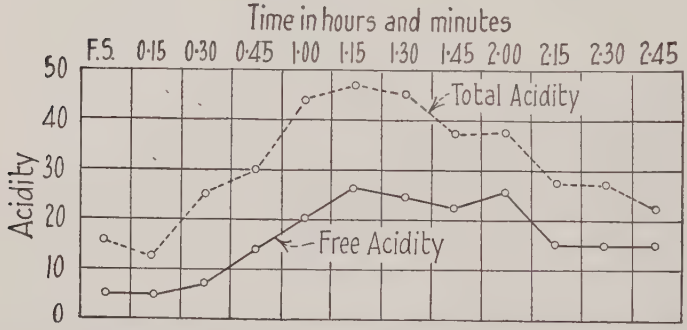


Fig. 153.—Diagram showing the average acidity of stomach fluid of 24 healthy persons studied by Talbot by the fractional method: F. S., Fasting stomach. (From Todd, Clinical Diagnosis by Laboratory Methods.)

a funnel. Use this filtrate for the following tests involving determinations of acidity. Place 10 mls. of the filtrate in a small Erlenmeyer flask and add about 5 drops of the indicator, phenolphthalein. Titrate, using 0.1 normal NaOH solution until the first appearance of a pink color; then add, with great care, drop by drop, the decinormal alkali until a definite pink has been obtained. Express the result in mls. decinormal alkali

[1] "I believe that frank macroscopic regurgitation of bile into either the fasting or digesting stomach is, with certain exceptions, an abnormal finding and suggests the disturbed physiology of the pylorus and the duodenobiliary apparatus or to pathology within the latter zone." Lyon, p. 440. The reader should refer to page 457 concerning these exceptions.

[2] This means the sum of all acid factors. As long as buffers exist in the gastric secretion, the *p*H does not rise above *p*H. 3.0. When buffer action is absent or destroyed then free acidity (*p*H 3.0–1.2) ensues. See Rehfuss, 1927.

necessary to neutralize 100 mls. of gastric secretion.[1] Protids, peptons, etc., act as buffers and lower the concentration of total titratable acidity. We shall discuss the meaning of variations in total acidity later (page 449).

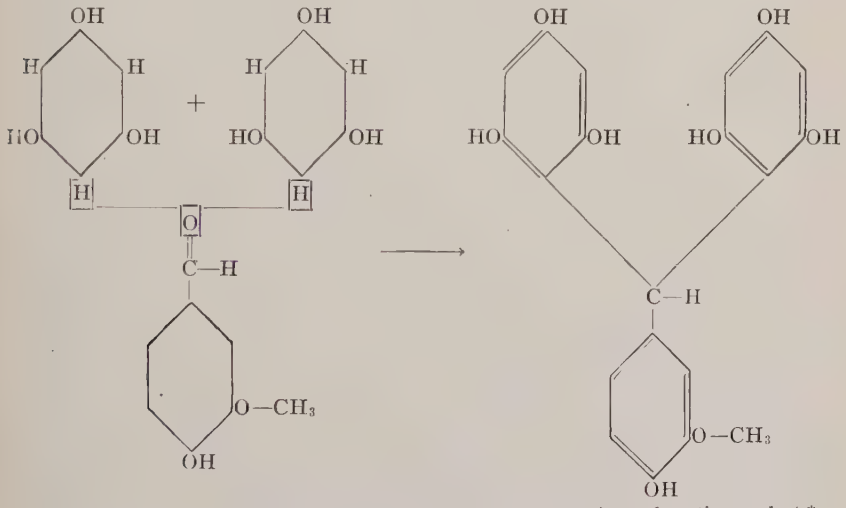
(2) *Free Acidity: Method of Günzburg.*[2]—Principle: In the presence of a low concentration of HCl ($4(10)^{-4}$ normal HCl or greater $\simeq$ *p*H 3.39), two indicators which make up Günzburg's reagent,[3] vanillin and phloroglucinol, condense together.

Two molecules of vanillin condense, with the elimination of a molecule of water. The color of the resulting compound is red. If HCl be absent, a brown color is developed, due to the oxidation of the

[1] In some hospitals it is customary to convert this reading into grams of HCl, multiplying by the decimal 0.00365 (1 ml. 0.1 normal alkali $\simeq$ 1 ml. 0.1 normal HCl; a normal solution contains 36.4 gs. of HCl per liter and each ml. contains a thousandth of that, or 0.0365 g., and a decinormal solution 0.00365 g. per ml.). Total acidity expressed in grams HCl per 100 mls. of gastric secretion varies normally between 0.14 to 0.26 g. HCl. However, the total titratable acidity involves more than HCl.

[2] Günzburg, A., German physician, died during the 19th century.

[3] Appendix. The chemistry of the reaction is as follows: The reagent consists of 2 per cent. vanillin and 4 per cent. phloroglucinol, alcoholic solution. Catalyzed by HCl in a concentration $\leqq$ 0.0014 per cent. ($\simeq$ *p*H 3.4) these two compounds condense to form a pyrocatechin substance, the formula of which is (the whole reaction is given):



Vanillin (meta-meth-oxy-para-oxy-benzaldehyde). With two molecules (upper) of phloroglucinol (1, 3, 5, tri-hydroxy-phenol).

Pyrocatechin condensation product.*

lactic and other acids of low ionization are negative.

* For an extensive account of the Günzburg Reaction see: Christiansen, J., Untersuchungen über freie und gebundene Salzsäure im Mageninhalt. I. Bestimmung freier Salzsäure im Mageninhalt. Biochem. Zeitschr., vol. 46, p. 24, 1912.

vanillin to form a pyrocatechin acid, protocatechuic acid, $C_6H_3.CHO-(OH)_2$. This color is likewise obtained if the preparation is overheated in making the test. There are two methods of making the test, the first being preferable:

Method 1: Fill a burette with 0.1 normal NaOH solution. Arrange to titrate this solution against 10 mls. of the filtrate from the intubation specimen as follows: Have ready a small porcelain evaporating dish and place in it 1 drop of Günzburg's reagent; lay the dish on a boiling water-bath. Now add, 1 ml. at a time, 0.1 normal NaOH from the burette to the 10 mls. of gastric filtrate in the beaker; mix and add 1 drop to the evaporating dish. Continue until you no longer obtain the carmin color of the drops in the dish. Take the reading of the burette. This gives the number of mls. of 0.1 normal alkali necessary to neutralize the free acidity of the specimen. Record as for total acidity.

Method 2: Dilution method. Proceed as in Method 1 above with the evaporating dish, but add, directly, a drop of the gastric secretion to the drop of reagent in the dish on the water-bath. If HCl is present in amounts greater than 0.0014 per cent., the red color develops. Now make a known dilution of one volume of gastric juice and repeat the test with 1 drop of this preparation. Continue, recording the amount of dilution until the red color is hardly perceptible and beyond which it is not observed; this is the end-point.

Calculation: Suppose you diluted the filtrate of gastric juice five times before the red color disappeared; then one-fifth of the total volume of the sample contains 0.0014 per cent. or 0.0004 normal HCl. Five times that amount gives the concentration in the sample taken. Express the results in mls. 0.1 normal HCl per 100 mls. of the juice.

Combined Acidity.—This fraction of total acidity is determined by difference: From the figure representing total acidity subtract free acidity; the result is the bound acidity.

Buffer Value.—This value may be determined by titrating with decinormal alkali, thymol blue (acid range) as indicator. *Procedure:* Titrate 10 mls. gastric contents with 0.1 N. NaOH and 10 drops (0.2 ml.) of a 0.2 per cent. thymol blue solution to the full blue color (pH 9.6); this gives the full buffer value. Express in mls. 0.1 N. alkali or acid necessary to neutralize 100 mls. gastric secretion. Example: 4.4 mls. of gastric secretion neutralize 2.28 mls. 0.1 N. alkali. Then 100 mls. secretion = 50.1 mls. decinormal acid. Suppose the free HCl amounted to 19.5 mls. 0.1 N. acid; the buffer value

is 50.1 − 19.5 = 30.6 mls. 0.1 N. alkali, the buffer value. If no free HCl is present obviously the subtrahend is zero; or, it may be less than zero, that is, the reaction is alkaline. The acid deficit may be determined by titrating with 0.05 N. HCl to free acidity (*p*H 3.0).

Hydrogen-ion Method for Free Acidity.[1]—Since gastric acidity is usually determined for persons in whom a pathological condition is suspected, and since, as we have stated on page 435, such conditions commonly involve a lowering of the gastric acidity, the methods just given are inaccurate. However, in many cases, they give reliable results if the acidity is high or normal. The only exact method is that of the determination of hydrogen-ion concentration, either by the electrometric method[2] or by the colorimetric procedure; this last we shall give here:

Sørensen Colorimetric Method for Determination of Free Acidity.[3]—Principle: A preliminary test to determine the general reaction of the sample is made; then an appropriate indicator is added. The particular hydrion concentration is indicated by the color acquired by the solution when the indicator is added. Procedure: Exploratory test: Using litmus paper as an indicator, determine the general reaction. If the paper is neutral or alkaline, the determination of free acidity is, of course, useless. If the reaction is given (blue to red), indicating acidity, then add to about 2 mls. of the gastric secretion in a test-tube one or more drops of thymol-blue-acid-indicator (*p*H 1.2 to 2.8). If a yellow color is given, with no orange tinge, the *p*H lies in a lower acid range; if the color is red to orange, the range is within that just given for thymol-blue-acid-range (about *p*H 2). Then, using 10 mls. in a test-tube with 2 or 3 drops of indicator, compare the color developed with that of the standard phthalate-HCl mixtures obtained from the side desk. If the range falls below that of this indicator, then repeat the above procedure using 2 mls. of the gastric juice in a test-tube and add 1 drop of the indicator brom-phenol-blue (*p*H range 3 to 4.6). Here the color must be either tan or purple. If within this range, make the appropriate comparison of the color developed in 10 mls. of gastric secretion treated with one or more drops of the brom-phenol-blue, with that of the standard

[1] For the difference between titratable acidity and hydrion concentration see page 87.

[2] Pages 53 and 65.

[3] For an excellent discussion of gastric acidity see the papers of Shohl in the Johns Hopkins Hospital Bulletin, vol. 31, page 158, 1920.

solution on the side desk. Continue in this manner until you have located the appropriate indicator and determined the proper hydrion concentration, using, if necessary, the indicators methyl-red, brom-cresol-purple and brom-thymol-blue. The average normal hydrion concentration of human gastric juice under the conditions of intubation given above is *p*H 1.7.[1]

Test Paper Method of Denis and Silverman.[2]—Following a suggestion of MacClendon, test-papers are made as follows: By means of 95 per cent. ethanol, make a 0.5 per cent. solution of the indicator dimethylamino-azobenzene and secondly one of thymol blue acid range of 0.1 per cent. concentration. Procure two sheets of filter paper (Whatman No. 044, Munktell No. 00 (Arthur H. Thomas No. 5168), or Carl Schleicher and Schüll No. 590), lay them, one at a time in a 200 mm. dia. evaporating dish and flood with the dye solution. Then drain off the excess dye and dry the paper before a fan or blower. Cut the papers into strips, keeping the two kinds separate. Store in a stoppered salt-mouth bottle. *To make the test* dip one end of a piece of the dyed paper into the gastric secretion and compare the color with that indicated below:

	*p*H.	Color.	Mls. 0.1 n. alkali.	Condition.
Dimethyl-	1.4	Red	44.0	Hypernormal
amino-	1.6	Reddish orange	27.0	Normal
azo	1.8	Orange	17.0	Normal
Benzene	2.0	Yellow-orange	10.0	Hyponormal
Thymol	2.0	Purple-red	10.0	Hyponormal
blue	2.4	Pink (faint)	4.0	Hyponormal
Acid range	3.0	Yellow	1.0	Anacidity.

By "mls. 0.1 n. alkali" is meant the number of mls. of decinormal alkali necessary to bring to *p*H 7 (neutralize) 100 mls. of a solution of the normality given. Thus, if the color is faint pink with thymol blue, 4.0 mls. of 0.1 n. NaOH would neutralize 100 mls. of solution the *p*H of which is 2.4. The test-papers may be checked against Sørensen or other buffer solutions (pages 66 and 898ff.).

Acidity Curves.—If the acid concentration determined for stomach contents as above be plotted with ordinates representing acidity and abcissæ, time, it is possible to make an accurate study of normal

[1] If it is necessary to convert *p*H into C_H, see page 42. This is easily accomplished by a slide-rule, such as the MacLean rule with slide reversed (Chapter XVI), or, better, by means of a duplex rule (Fig. 14).

[2] Denis, W. and Silvermann, D. N., Amer. J. Med. Sci., vol. 169, page 25, 1925.

and pathological conditions. Although such studies on the normal stomach show all gradations from achylia to "hyperacidity,"[1] curves of acidity, when considered with other factors (blood, bile, lactic acid, etc.), are of value clinically. The following *types of curves,* however, are recognized:

(1) *Normal curve:* This is an isometric curve, the peak occurring during the fourth period of intubation, IV.[2]

(2) *Achylia gastrica:* This curve is flat, parallel with the abscissa line.

(3) *Hyperacidity curve:* This curve rises throughout the eight periods, due to a stimulus of nervous origin (vagotonia, or abnormal sensitivity of the vagus nerve), duodenal ulcer, appendicitis, etc.

(4) *Plateau Curve: This curve resembles* (2), but a certain degree of acidity is developed and secretion takes place continuously; this curve is known as the plateau curve. It is due to an interference with the escape of the gastric secretion from the stomach, as in pyloric stenosis, or hardening of the pyloric sphincter.

(5) *Trauma Curve: This curve resembles* (1), *but the apex is much higher* and is reached within the second period, II. This is the curve of trauma, produced by the hyperesthesia of the subject during the intubation. A condition of achylia is presented toward the end of the period due to the same cause.

Detection of Lactic Acid as an Index of Fermentation of Glucids by Bacteria[3] Due to Decreased Secretion of Hydrochloric Acid.—*Hopkins' reaction:* Place 5 mls. of filtered gastric secretion in a test-tube and add 1 volume of ether. Shake the contents of the tube and then wait until the zones separate. By means of a pipette siphon off the lower layer, leaving the ether layer, which contains the lactic acid. Place the tube in a boiling water-bath to remove a large portion of the ether. Cool the tube and add 5 mls. of concentrated sulphuric acid and 3 drops of a 40 per cent. cupric sulphate solution. Agitate the contents and leave in the bath for several minutes. Cool and add

[1] "The clinician must henceforth take cognizance of the fact that hypersecretion and clinical hyperacidity as well as hypo- and anacidity are not only compatible with health, but are found in considerable percentage of normal individuals," Carlson, A. J., p. 29. "Studies of healthy men indicate that there is no acidity in disease which cannot be encountered in health," Rehfuss, M. E., Jour. Amer. Med. Assoc., vol. 71, p. 1534, 1918.

[2] The periods of withdrawal (fifteen-minute intervals) are designated by Roman numerals; thus at the end of the first hour, when the normal acidity curve reaches its peak, we have Period IV.

[3] Such as Sarcina ventriculi and S. fuscescens.

about 2 drops of 0.2 per cent. alcoholic thiophen[1] solution. Mix and leave to warm in the bath. The reaction is positive if a cherry-red color appears.

Detection of Lactic Acid by Uffelmann's Ferric Chlorid Test, Folin's Modification.—Refer to page 378. Place 5 mls. of the filtered gastric secretion in a test-tube and add 3 drops of a diluted (1:20) HCl solution; then add 1 volume of ether and stopper the tube with your thumb. Invert the tube slowly and right it again, permitting the ether fumes to escape by the withdrawal of the thumb. Remove the lower aqueous layer as in Hopkins' test, and add drop by drop a special dilute solution of ferric chlorid,[2] agitating the contents until a clear yellow color has developed, if the test is positive. A concentration of water retards the reaction. The test is more specific than the older Uffelmann test, which responds to other organic hydroxy-acids, like oxalic, tartaric, etc. The reaction resembles Fehling's reaction in that a metal hydroxid, the brown ferric hydroxid ($Fe(OH)_3$) in Uffelmann's reaction, becomes reduced to ionic condition by increased ionization during the hydrolysis of the chlorid:

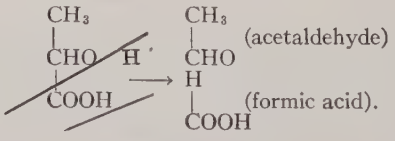
$$3H_2O + 2FeCl_3 \longrightarrow Fe^{+++} + 3Cl^- + H^+ + OH^- + Fe(OH)_3 + 3HCl.$$

The ferric ion, Fe^{+++}, is almost colorless, while the hydroxid in contact with the chlorid is brown to yellow, depending upon the degree of ionization.[3]

Quantitative Methods for Determining the Peptic Activity of the Stomach.

The Method of Roaf.—Blood fibrin has been stained with Congo-red[2] solution, washed, etc. Place a small amount (about 0.5 g.) of this dried, stained fibrin in contact with 15 mls. filtered gastric secretion and 5 mls. of 0.3 per cent. HCl solution. Note the time. Leave at 50° C. until digestion has occurred; this stage is indicated when the solution becomes the color of the dye. The course of digestion may

[1] Page 378. The reaction is as follows:



Acetaldehyde and formic acid are formed in the presence of H_2SO_4 and $CuSO_4$ (acting as catalyzer). The formic acid becomes reduced to formaldehyde, and the two aldehydes, acetaldehyde and formaldehyde, then react with thiophen to form condensation products. See page 117 and also page 378.

[2] Appendix.

[3] For Uffelmann's reaction proper see page 378.

be followed by matching the color of the free dye against a standard solution of Congo-red, 0.01 g. per 100 mls. of water.

The Method of Gross.[1]—Secure 10 test-tubes and arrange them in a rack. To the first add 0.1 ml. of the filtered gastric secretion; to the second, 0.2 ml. and so on, increasing 0.1 ml. for each tube. Use the 1-ml. Mohr pipette which is graduated in tenths. Now add to each tube 10 mls. of the special casein[2] solution. Place the tubes in a bath at 40° C. and leave exactly fifteen minutes. Then add to each tube 3 drops sodium acetate solution[3] to cause the precipitation of the undigested casein. The smallest quantity of pepsin solution necessary to digest the 10 mls. of casein is the figure used to designate the activity of the pepsin. One unit is defined as that amount of pepsin in 1 ml. of gastric secretion which will cause the digestion of 10 mls. of casein solution. If in the above Exercise it was found that the tube containing 0.5 ml. of pepsin solution showed the smallest amount of pepsin necessary to cause the digestion of the casein, then there are $\frac{1.0}{0.5} = 2$ units of pepsin in the gastric secretion under examination.

The Mette[4] *Tube Method.*—Preparation of the tubes: The method is given in the Appendix. The determination: Place duplicate tubes in contact with 1 ml. of gastric juice, diluted with 10 mls. of 0.3 per cent. HCl solution. Place in a bath at 40° C. and leave over night or until the following period. Then wash the two tubes carefully with distilled water and dry by means of a piece of filter paper. Place one tube upon a glass slide under the low power of the microscope provided with a calibrated scale to read in millimeters and estimate the amount of egg-white digested. Estimation: This is done in terms of the suggestion of Schuetz,[5] that pepsin activity be expressed as the

[1] Gross, M., Roosevelt Hospital, New York.

[2] Pure casein should be used. Page 110, note. The preparation is made under the direction of Dr. Harris, formerly of the Department of Physiological Chemistry, Yale University, New Haven, Connecticut. The special solution is described in the Appendix.

[3] Twenty per cent. aqueous solution.

[4] Mette, E. L. P., German physician, died during the 19th century. The pronunciation, frequently incorrect, is "met'tah."

[5] Schuetz, E., University of Vienna, Austria (contemporary). See Zeitschr. f. physiol. Chem., vol. 9, p. 577, 1885. Schuetz suggested that the unit of pepsin be taken as that amount which will give 1 gram of pepton, the pepton being estimated by the optical rotation of the solution. The equation is:

$$P = \left(\frac{m}{4 \times 39.18}\right)^2 \times \frac{1}{p}$$

where P is the unit, m the observed rotation, each quadrant of which is 39.18 degrees of arc, which corresponds, when all four quadrants are taken (4), to 1 gram of pepton; p the amount of gastric secretion used. Briefly, we may interpret this to mean that two pepsin solutions stand together as the squares of the amount of egg-white digested.

square of the amount of egg-white digested. Hence, if a Mette tube be found to have digested from it 1.2 mm. of egg-white by the pepsin, then the activity of the gastric secretion is $(1.2)^2 = 1.44$ unit per ml. of secretion.

The Significance of the Total Amount of Gastric Secretion.—The total residuum of the stomach normally averages 50 mls.,[1] with variations from 30 to 180 mls. Pathological modifications of this average occur. Obstruction or factors delaying the emptying of the stomach increase the amount as well as the figures for total acidity. Disease, like carcinoma, affecting the body as a whole or parts of great importance cause a characteristic reduction in the amount of residuum as well as in total acidity. As a rule a residuum of more than 50 mls. coincident with high total acidity indicates the presence of gastric ulcer or of incipient carcinoma. However, nervous and functional disorders frequently give these high amounts. As an index of stomach motility gastric residuum is of importance, and the same may be said of gastric acidity figures. Indeed, as Carlson says: "The rôle of the gastric juice in the maintenance of health and in the etiology of disease has been exaggerated to the neglect of the importance of gastric mobility."[2]

Pepsin and Its Work.—In common with practically all enzymes there is but little accurate knowledge of the chemical nature of pepsin. We may summarize what knowledge we have as follows:

Physical state: Colloidal; salts out with $(NH_4)_2SO_4$.
Chemical relations: Protid.
Known components: Glucid, probably glucose.

Elementary analysis:	C	50 per cent.
	H	7 "
	O	23 "
	N	14 "
	Fe	0.1[3] "

Chemical properties: Extracted readily with acid.
Destroyed by alkali.
Heat stable to about 60° C.
Zymogen:[4] Pepsinogen.
Co-enzyme, or activator: Acid.
Products of action: Albumoses, peptons, and later peptids containing the biuret link; after prolonged action, amino-acids.
Destroyed in acid solution at *p*H 1.3 or above.

[1] This and other data concerning gastric digestion have emanated largely from the Laboratories of Physiological Chemistry of Jefferson Medical College under the direction of P. B. Hawk (now of 1440 Broadway, New York, N. Y.) associated with M. E. Rehfuss (page 440).

[2] Carlson, A. J., page 433.

[3] The phosphorus which is frequently included in an elementary composition of pepsin is due to the admixture of a phospholipid, like a lecithin. Ca and S are not affected by purification methods, showing their constant relations to pepsin.

[4] Greek *zyme*, yeast (refers to enzyme; see page 427), and *gennan*, produce.

The effect of pepsin upon different zymolytes varies with the different substances.

Thus, the optimum for pepsin working upon:

Casein	*p*H 1.8
Egg-albumin	*p*H 1.2–1.6
Gelatin	*p*H 3.2

Sørensen believes that the optimum *p*H for pepsin varies according to the length of time the enzyme acts, increased acidity being necessary as time progresses. *The optimum pH for pepsin is now believed to be the degree of acidity which most favors the union of enzyme with zymolyte.* This degree of acidity corresponds in cases studied critically, to the iso-electric point.[1]

Effect of Enzymes on Milk Protid.—Of the three milk protids (casein, lactalbumin and Osborne's alcohol soluble protid) casein is of greatest importance. Gastric juice does not split off phosphorus, while an enzyme to be considered later does (trypsin). Alkali (1 per cent. NaOH) easily cleaves phosphorus from casein. Of the enzymes mentioned, none, ordinarily, is specific for casein, but either pepsin under certain conditions, or a different enzyme chymosin, or rennin causes the coagulation of casein.[2]

Rennin or Chymosin.—We shall present some practical exercises on rennin and then discuss the relations of this enzyme to pepsin.

Exercise 6.—Place 10 test-tubes in a rack. Add 5 mls. of skimmed or separator milk to each tube. Then add to tube No.

(1) Nothing (control).
(2) Two mls. of the chymosin solution.[3]
(3) Two mls. boiled rennin.
(4) Two mls. rennin and 2 drops of 1:10 acetic acid solution.
(5) Two mls. rennin and 2 mls. 2 per cent. Na_3CO_3 solution.
(6) Two mls. rennin and 1 ml. potassium oxalate solution, 1 per cent.
(7) Two mls. rennin and 1 ml. potassium oxalate solution as above (Tube 6). Boil and cool. Add 1 ml. 1 per cent. $CaCl_2$ solution.
(8) Boil the contents of this tube. Cool. Add 2 mls. rennin solution.

[1] Herzmann, A. B. (University of Michigan), and Bradley, H. C., Jour. Biol. Chem., vol. 59, p. 19, 1924. See page 112.

[2] It is interesting that none of the enzymes capable of cleaving phosphorus in other compounds (bone phosphatase, kidney phosphatase, etc.) is capable of removing phosphorus from casein.

[3] Page 896.

(9) Treat as Tube 8; then add 1 ml. $CaCl_2$ solution.

(10) Treat as Tube 8; then add 2 drops 1:10 acetic acid.

The tubes are placed in a thermostat at 40° C. and the time noted. The tubes are watched carefully, and in order to determine the coagulation time it is necessary to lift the tube from its support, for merely observing the appearance of the preparation as it stands seldom is accurate enough.

From the above experiment it is evident that:

(1) Spontaneously fresh milk does not coagulate at body temperature.

(2) The addition of rennin accelerates coagulation.

(3) Rennin is heat labile and is destroyed by boiling.

(4) The addition of acid leads to accelerated coagulation, due to the solution of calcium salts necessary for the precipitation of the coagulated protid.

(5) The process of coagulation is inhibited by alkali.

(6) Reagents which render calcium insoluble, like oxalates, forming insoluble calcium oxalate, inhibit coagulation.

(7) The inhibitory power of the oxalate may be counteracted by adding an excess of calcium, as in $CaCl_2$, and also that rennin exerts its action in the absence of calcium, the clotting (precipitation) becoming evident when Ca is added.

(8) Boiling the milk before adding rennin causes the appearance of a very solid, tough curd, if any.[1]

(9) The addition of $CaCl_2$ to such a preparation as (8) induces coagulation.

(10) The addition of acid favors coagulation of boiled milk.

Practical Suggestions.—All enzymes work at the exposed surfaces.[2] The smaller the volume and the relatively larger the surface, the greater will be the enzyme activity. Similarly, in the case of rennin, the clot will digest more rapidly if it is not a large solid one. In children it is sometimes desirable to keep the curds small and flocculent by the addition of a little lime-water, as alkali. Again, in an opposite way, if an alkali, like sodium bicarbonate, be administered to a patient with gastric ulcer and then milk in some form, the milk remains uncoagulated and will not digest. The lactose will

[1] Boiled milk is not equivalent in diet to unboiled milk, owing to the loss of soluble calcium. (See Magee, H. E. and Harvey, D., Biochem. Journ., vol. 20, page 885, 1926.)

[2] Or within if the enzyme is soluble in the material upon which it is working. A lipase digests fat only if it is dissolved by the fat.

undergo fermentation and gas will accumulate, giving rise to discomfort, if not to a serious condition.[1] Since rennin, like other enzymes, works according to the "R. G. T." rule,[2] that is, that enzyme action increases about two to three times for every 10° C. rise in temperature the drinking of cold milk tends to cause the production of smaller curds.

The production of rennin varies directly with that of pepsin. Whatever pathological factors affect pepsin, affect rennin also. Thus the possibility of their identity arises.

Are Rennin and Pepsin Identical?—In answering this question[3] we may say:

(1) Rennin and pepsin run parallel in quantities when estimated during the secretory period following a meal of protid. This has been shown both by the estimations of the content of pepsin and rennin in gastric mucosa and in the gastric secretions both of the normal stomach and the Pavlov[4] stomach in experimental animals.

(2) The deterioration of peptic and rennin activity in a piece of gastric tissue kept in an incubator for some time runs parallel in the two cases, as it does in a juice brought to 60° C.

(3) Rennin-like action is found universally in plants and animals where the action is correlated with the presence of a protidoclastic enzyme.

(4) The action of rennin resembles the initial action of any protidoclastic enzyme, namely, to cause the cleavage of the protid molecule.

(5) If the gastric secretion be dialyzed through a membrane, or through a Berkefeld filter,[5] rennin is affected in such a manner that it may neutralize an antirennin, but it does not cause milk coagulation. The antirennin is contained in blood-serum. Pepsin does not show these relations.

(6) Rennin requires the presence of calcium in order that it may coagulate milk. Pepsin, or the peptic reaction, works in the absence of Ca, although it is accelerated by Ca even to a greater extent than rennin.

[1] By inflation the stomach is displaced and the coronary circulation in the heart is partly occluded.

[2] From the German "Reaktionsgeschwindigkeits-Temperatur," a thermochemical term due to van't Hoff (see page 177, note 4).

[3] For a brief discussion of this question see Edie, E. S., Biochem. Jour., vol. 15, p. 507, 19.

[4] Page 439.

[5] A cylinder composed of the fossil remains of shells of one-celled organisms called diatoms.

(7) Gastric secretion when kept constantly at 40° C. loses its rennin action, but retains its protidoclastic properties.

(8) Gastric secretion treated with a precipitant, like lead acetate, loses its peptic action, but retains its rennin action.

(9) The optimum hydrion concentration of pepsin is *p*H 1.7, and the lowest *p*H where the peptic function of gastric secretion is evident is *p*H 5, whereas, the optimum for rennin action is *p*H 6.8, but the latter action occurs even at *p*H 7.6, that is, in an alkaline medium.

(10) A comparison of the rennin and peptic activity of young and adult mammalian gastric secretions shows the following results (rabbit):

	Average milk coagulation time.	Fibrin digests.
Young	Eighteen minutes	None after two hours
Adult	None after two hours	10.8 mls. 0.1 n. nitrogen

Of the foregoing data the first four points indicate the identity of rennin and pepsin, while the remaining six argue against such a possibility. It is probable that the following explanation is valid: (1) In the young rennin, a special milk-curdling, hydrolytic enzyme is present in greater amounts because milk is the sole or larger part of the diet. The average normal *p*H of the gastric secretion of the young mammal is *p*H 3.5 to *p*H 5, which is below or at the minimal reaction for peptic activity, but which is adequate for the action of rennin. (2) Pepsin resembling all protidoclastic enzymes has a milk-curdling action varying in degree with such factors as hydrion concentration, temperature, and zymolyte. Milk will undergo coagulation in an acid medium free from any enzyme.[1]

The Manner of Action of Rennin.—This may be summarized as follows:

(1) Hydrolysis of casein occurs similarly as in protid hydrolysis in general.

(2) The product of hydrolysis is a protidtemn, or group of such substances of the nature of albumoses and also a product which unites with calcium to form a calcium-caseinate, which is insoluble near its iso-electric point (about *p*H 6.7).

(3) The precipitation of calcium-caseinate incorporates the fat globules and this mixture is known as the "curd."

(4) The protidtemns do not enter the curd, but remain in the watery solution left after precipitation, as a whey albumose.

[1] The buffer effect of different milks is an important point. Thus, to bring 100 mls. of human milk to a *p*H 5.0, 18 mls. of decinormal acid must be added, while in the case of cow's milk, 55 mls. must be added.

The Fate of the Curd.— The curd undergoes hydrolysis into protidtemns—albumose, pepton, peptids, and amino-acids. The process of gastric digestion is supplemented by tryptic and ereptic digestion in the intestine. Absorption of the products takes place in the intestine. The phosphorus cleaved from casein, along with the other forms of phosphorus in milk, namely, inorganic and, thirdly, loosely-combined with casein, enters the blood.[1]

The Fate of Fat in the Stomach.—Fat in finely divided amounts (emulsified) undergoes some gastric hydrolysis, while fat in larger aggregates is not affected in the stomach.[2] Emulsification of the fat takes place to a small extent by the combined action of the heat of the stomach (37° C.) and of the acid, HCl, but in addition to this rather insignificant action, emulsified[3] fat is hydrolyzed to glycerol and fatty acids which may become soaps. This hydrolysis is effected by a specific fat-cleaving enzyme, gastric lipase, which is secreted in the gastric mucosa. This process of fat digestion, however, is insignificant when compared to the digestion of fat in the intestine. The emulsification of fat in the stomach is largely a matter of the action of bile-salts which are regurgitated from the duodenum. Since this process is one dependent upon an unusual, rather than an ordinary function[4] of the pylorus, the emulsification of fat is of an exceptional and not usual procedure in the stomach. The presence of a gastric lipase is probably an adaptation of the organism to economy; regurgitation of the bile occurs mechanically with a consequent physical change in the fats which then undergo hydrolysis if a hydrolyzing agent be present. Such an agent is supplied by the gastric lipase.[5] The fact that gastric lipase is destroyed in HCl 0.02 (one-tenth the normal acid concentration in the gastric secretion) shows that the physiological importance of this enzyme is small. On a milk diet, as in the young, when the milk serves as a buffer to lower the Ch, the rôle of gastric lipase is enhanced.[6] Aside from this lipase and that secreted by the pancreas (steapsin) all other esterases in the body

[1] Kay, H. D., Biochem. Journ., vol. 19, page 433, 1925.

[2] Since fat in the stomach frequently causes regurgitation of duodoenal contents containing steapsin, the fat as Willstätter (page 433) has shown is digested by this "foreign" enzyme.

[3] The reason why emulsified fat is hydrolyzed is given on page 118, note 1.

[4] See page 444 concerning the presence of bile in the stomach.

[5] A parallel instance of the economic utilization of enzymes may be cited: Erepsin of the intestine acts supplementarily to the gastric and duodenal protid-cleaving enzymes, pepsin and trypsin. Protids which have escaped the action of these enzymes are hydrolyzed by the erepsin.

[6] For a consideration of the rôle of lipases in the organism see Willstätter (page 133).

hydrolyze esters of lower fatty acids (not triglyceride of stearic, palmitic and aleïc acids).

Diglucids, like maltose, sucrose, and lactose, are not hydrolyzed in the stomach, for no enzymes are detectable in gastric secretion capable of catalyzing their hydrolysis. If these glucids are retained for any length of time, however, the increased acidity of the gastric secretion resulting from the continuous production of acid over the period cause some hydrolysis, but this is physiologically insignificant.

INTESTINAL DIGESTION

The Enzymes of the Intestine.— In general it may be said that the intestine has a suite of enzymes adequate for the digestion of any food material. They are:

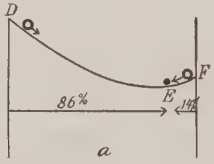


Fig. 154.—Law of mass action. Equilibrium of opposing reactions, the vigor is indicated by the height of the lines *D* and *F*, and the velocities by the arrows. The graph represents the equilibrium maltose $\overset{\text{maltase}}{>}$ glucose. (From Holland, Medical Chemistry and Toxicology.)

Glucidases, enzymes hydrolyzing the glucids ("carbohydrates").

Diglucidases:

Maltase, α-glucosidase hydrolyzing maltose to two molecules of glucose.

Lactase, hydrolyzing lactose to glucose and galactose.

Sucrase, hydrolyzing sucrose to glucose and fructose.

Glucosidases (other than above): β-Glucosidases hydrolyzing glucosides, like amygdalin.[1]

Polyglucidases:

Amylase, hydrolyzing starch to maltose.[2]

Lipidases,[3] enzymes hydrolyzing neutral fats and substances resembling the neutral fats, phospholipids, esters, etc., to

[1] The presence of an emulsin-like enzyme capable of hydrolyzing amygdalin, a glucoside containing the group CN, has not been demonstrated in intestinal secretions, but when pure amygdalin free from emulsin of plant origin is taken internally symptoms of HCN poisoning have been observed leading to the necessity of explaining how this glucoside is split up, freeing HCN.

[2] Also called amylopsin, from the Greek *amylon*, starch, and *opsis*, appearance.

[3] Called also "steapsin" from the Greek *stear*, fat.

glycerol, fatty acids, nitrogenous constituents, phosphoric acid, etc., according to the kind of fat.

Lipase, hydrolyzing neutral fats to glycerol and fatty acids.

Phospholipidase, hydrolyzing lecithin and other phospholipids.

Esterase, hydrolyzing the lower esters to an alcohol and acids.

Protidases.—Enzymes hydrolyzing substances composed of α-amino-acids to these units.

Trypsin, hydrolyzing native protidtemns, or even the protids themselves to amino-acids.[1] The action of trypsin is supplemented by:

Erepsin, hydrolyzing protidtemns to amino-acids. Protids are not affected by this enzyme.[2] It is probable that this enzyme is not identical with the "autolyzing" enzyme of all tissues. The enzyme is produced in part in the pancreas and in part in the intestinal wall.

In addition to the enzymes concerned with glucids, lipids, and protids there are certain enzymes concerned with *oxidation*, *reduction*, and *deaminization*, functions which are common to all tissues. How far these may be considered alimentary enzymes cannot be said. We include them, however:

Catalase (Loew) decomposes hydrogen peroxid.[3] The nature of this enzyme if indeed it is a true enzyme, is not clear.

Peroxidases affecting organic peroxids. We have shown[4] that the "peroxidase" of the blood is, in reality, Fe. It is possible and even probable that a similar condition holds respecting the "peroxidase" of the intestine. Recent work has shown the dependence of oxidizing functions of the di-peptid glutathion[5] upon traces of iron.

Aldehydase, which is present probably only in cells contained in the intestinal juice. It has not been demonstrated in the pancreas.

Tyrosinase, oxidizing tyrosin, forming "melanins."[6] It is of the nature of a peroxidase.

Deaminase.—This is of doubtful occurrence, although intestinal secretion causes deaminization, which may be of bacterial origin.

[1] When some protids are subjected to the action of trypsin, a few of the constituent amino-acids are split from the molecule. Thus, tyrosin and tryptophan leave the molecule readily; others, like leucin, alanin, glutamic and aspartic acids, are less readily cleaved, while phenyl-alanin is not split off from the molecule.

[2] See Cohnheim, O., Zeitschr. f. physiol. Chemie, vol. 33, p. 451, 1901; vol. 35, p. 134, 1902.

[3] Page 100.

[4] Page 118.

[5] Page 343. Insulin seems to depend upon the presence of sulphur.

[6] Page 266.

The following exercises demonstrate the action of intestinal enzymes, Exercise 7 showing the necessity of a zymogen, enterokinase in tryptic digestion:

Exercise 7.—Obtain from the store-room 10 mls. each of the casein digests which have been prepared as follows:

(*a*) Casein + suspension of ground pancreas.

(*b*) Casein + suspension of ground pancreas + ground mucosa of the intestine.

Determine by formol titration[1] which case of digestion has proceeded to the greater extent.

Exercise 8.—*The Rennin-like Action of Pancreatic Secretion.*—Method of Cole:

(1) Preparation of special calcified milk: Place 50 mls. of fresh whole milk in a 100-ml. volumetric flask and add 10 mls. of 5 per cent. calcium chlorid solution. Make up with distilled water to the 100-ml. mark, mix, and use for the following experiment:

(2) The Experiment: Place 3 clean test-tubes in a rack. A is control, B and C are experiments. To each add 5 mls. of the special milk. To A add nothing; to B add 1 ml. of the pancreatic solution; to C add 1 ml. of the pancreatic solution which has been brought to the boiling-point and then cooled to room temperature. Place the tubes in a bath at 40° C. Examine at intervals, and if there is no clotting in tubes A or C, whereas clotting occurs in B, a positive rennin-like action is demonstrated for the pancreatic secretion. If, however, either or both controls are coagulated, the experiment must be discarded; moreover, the experiment must be followed carefully, the tubes being observed at frequent intervals, for in Tube B digestion of the clot may take place, making it appear that clotting has not taken place. This rennin-like action may be due to intestinal erepsin or to pancreatic trypsin.

Exercise 9.—*The Claude Bernard Test for Distinguishing Gastric and Pancreatic Digestion.*—Filter into separate test-tubes each of the following solutions:

(1) A gastric digest[2] made as described in Exercise 29 (page 235).

(2) A tryptic digest (Tube B, if positive, may be used after being left in the bath for twenty-four hours or longer).

[1] Page 291.

[2] The laboratory should maintain a gastric digest for this Exercise; or the student may set up in a test-tube such a digest and the Exercise completed in the following period.

To Tube 2 add 2 drops of glacial acetic acid; to each tube, then, add, drop by drop, bromin water,[1] noting any change of color in the solutions. The color may be caused to disappear if an excess of bromin water is added, but by adding half a volume of amylol and agitating the solutions, the tryptophan-bromin compound is absorbed and appears as a reddish or violet colored solution floating at the surface.

The principle of the test is that in tryptic digestion amino-acids are formed among which is tryptophan. This is why color appears when bromin is added.

The Difference between Erepsin of the Intestine and Tissue "Erepsin."—Alimentary erepsin differs from trypsin in regard to the action on simple protids, for trypsin hydrolyzes casein, egg-albumen, etc., while erepsin acts only upon the products of gastric or duodenal digestion, that is, peptons and higher peptids. Each tissue has an enzyme which resembles, in a general way, erepsin, but which differs from it in two particulars: (1) Reaction at optimum and (2) substances acted upon:

	Optimum.	Zymolyte.
Alimentary erepsin	7.8	Protidtemns.
Tissue "erepsin"	5.0	Simple protids, together with protidtemns.

It is probable that the autolyzing enzyme, called above "tissue erepsin," is not a single one, but a composite of two or perhaps more.

Exercise 10.—*Autolysis of Liver.*—Secure from the store-room 20 gs. of liver which has been ground in a meat-grinder and then sieved through a fine-meshed sieve. Place the mass in your 100-ml. volumetric cylinder, add 5 mls. of toluene, and make up to 100 mls. with distilled water. Now mix thoroughly and divide into four parts, 25 mls. each to a large (200 mm. by 20 mm.) test-tube. Label A, B, C, and D. A is control; to B add 5 mls. of 1:10 HCl solution. To C add 5 mls. of 1 per cent. Na_2CO_3 solution. To D add nothing until the following period; then add 5 mls. 1 per cent. Na_2CO_3 solution. Leave the tubes at 37° C. in a thermostat until the following period and examine for amount of digestion. Filter 5 mls. and make formol titration on the filtrates.

Autolysis and Atrophy.—There are many phenomena in human physiology in which the tissues undergo shrinkage in size and alterations in chemical composition. This process is attributed in some instances to the work of tissue enzymes capable of hydrolyzing pro-

[1] See Appendix.

tids, the so-called "autolytic" enzymes. The normal process of involution of the uterus immediately after the birth of the child during which the distended uterus shrinks to the size of the non-pregnant one is cited as an example of autolysis. A part of this process involves a loss of water and consequent reduction in size, but there is also a loss of substance. Again, in the disease of the liver known as acute yellow atrophy and in its artificial counterpart, phosphorus poisoning, there is a change involving the protids and fat. Chloroform when used in anesthesia causes lesions in the liver not unlike those produced by phosphorus. In all such instances the liver cells are poisoned to a greater or less extent and yet the tissue protidoclastic enzymes, especially in the acid medium, remain active. The acidity is produced by interference in oxidation (anoxemia), due either to lack of oxygen or to a loss of oxidizing powers of the tissues. Again, the resolution of exudates, like the pulmonary exudate following the crisis in that disease is considered a function of these enzymes.

The "autolytic enzymes" are not derived from the alimentary trypsin or erepsin. The autolytic enzymes are resident in the tissues, and although they resemble the alimentary enzymes, they are quite distinct in origin and action.

The Fate of Trypsin; Its Utilization as a Functional Test for Pancreatic Efficiency.—If the motility of the stomach and intestines is normal, the protid-cleaving enzymes, pepsin and trypsin, are not present in the feces. But in diarrhea and diseases in which increased motility of the digestive tract occurs, trypsin especially and sometimes pepsin may be demonstrated. If it is desirable to test the efficiency of the pancreas in producing trypsin, two methods are possible: (1) Aspiration of the duodenal contents through a Rehfuss tube, using any of the tips employed in gastric analysis, like the Lyon tip; (2) administering a mild cathartic, like cascara sagrada. The increased motility which results causes the propulsion of the contents of the intestine carrying with them the trypsin which has not had time to become destroyed by the action of the other enzymes. The procedure for administering the tube and analyzing the intestinal contents thus obtained is outlined herewith:[1]

DEMONSTRATION.—*Intubation for Duodenal Secretion.*—Have the subject swallow the tube as for gastric analysis,[2] letting it descend to the second mark on the tube. The passage of the tube into the duo-

[1] This Exercise should be presented as a demonstration to students, one of their number offering himself as subject, as shown in Fig. 155, page 463.

[2] Page 441.

denum is facilitated by pouring 120 mls. of warm distilled water into the funnel of the tube. After this the subject lies on a couch in a "right Sims' position,"[1] while the tube is swallowed to the mark. This takes about twenty minutes after the tube has reached the stomach. Slow swallowing movements aid in the passage of the tip of the tube through the pylorus.[2] The contents of the duodenum are aspirated by means of the glass syringe which accompanies the tube (Fig. 151).

Fig. 155.—Duodenal tube. Collection of bile, A, B, and C portions, as shown by the bottles. Microscopic examination is being made for cellular elements, evidences of concretions, etc.

Analysis of the contents for trypsin: Strain the secretion through glass-wool, muslin or cotton, and filter through filter-paper. The analysis is made according to the principle used in the Gross method for gastric analysis:[3] To 5 mls. of the filtrate add 15 mls. of 1: 1000 Na_2CO_3 solution; filter. Set up two Erlenmeyer flasks and introduce 10 mls. of the filtrate into each. Then add 100 mls. of the special pancreatic casein solution.[4] Leave in the thermostat at 37° C. until the following period and treat as follows: Add to each flask, drop by

[1] See proper position in American Illustrated Medical Dictionary, 13th ed., Philadelphia, W. B. Saunders Co., 1925, p. 917. Also Fig. 155.

[2] With reasonable care as to the time limit, there should be no danger of the tube being thrown into loops in the stomach. If a fluoroscope is available, the tip may be located.

[3] Page 451.

[4] See Appendix, noting that the gastric and pancreatic casein solutions are not the same.

drop, a solution of 1 per cent. acetic acid. *If a precipitate occurs*, it is indicative of the *absence of trypsin*, since undigested casein is precipitated.[1] On the other hand, if digestion has occurred, due to trypsin, precipitation will not take place.

Trypsinogen and Enterokinase.—Trypsin is produced in the pancreas in an inactive form, known as trypsinogen. In Exercise 7 we have seen that the presence of intestinal mucosa is necessary for the functioning of the pancreatic secretion. Pepsin, we have seen,[2] must be activated by means of acid. The agent causing the activation of trypsin is known as enterokinase,[3] a substance of unknown chemical relations. It must be understood that trypsin will digest protid[4] and protidtemns without the addition of enterokinase, but the addition of enterokinase speeds up the reaction to such a degree that, comparatively, the digestion in the absence of enterokinase is negligible.[5] Enterokinase occurs not only in the mucosa of the intestine but also in leucocytes; and during autolysis in the pancreas (whether in the body or excised) kinase is formed capable of activating trypsinogen. Certain pathological states, pancreatitis and fat necrosis of the pancreas, have been attributed to the activation of trypsinogen while it is in the pancreas, owing (1) to the migration of enterokinase from the duodenum up through the bile-duct; (2) to the process of autolysis, or (3) to kinase secreted by leucocytes. However, the activating effect of the last is small. It has been demonstrated recently that calcium is necessary for the conversion of trypsinogen into trypsin.

The Activator of Pancreatic Secretion.—In order to avoid common misconceptions it may be well to introduce here a statement concerning secretin, which plays a rôle in the pancreas parallel to that performed in the stomach by the gastrins. Secretin accelerates the secretion of the total pancreatic product, but does not serve as an activator for any of the enzymes. Chemically, secretin has been identified with histamin,[6] a derivative of histidin, but pure preparations of the substance thought to be secretin, by the British pharma-

[1] See page 410 for the method of precipitating casein from milk.

[2] Page 434.

[3] From the Greek *enteron*, intestine, and *kinesis*, motion. Enterokinase has nothing to do with the motility of the intestine, but with the activation of trypsin.

[4] Page 459.

[5] Trypsin retards the action of trypsin, a function which is present even after the inactivation of the enzyme as a protid-cleaving enzyme, for when the solution is cooled and brought into contact with a protidtemn-trypsin solution, like gelatin and trypsin, the action of the last is inhibited.

[6] Page 273.

cologist Dale,[1] do not give the typical effects of secretin when introduced into the blood system. The fact remains, however, that there is some substance existing in the mucous membrane of the duodenum and jejunum which, when activated by hydrochloric acid, then neutralized and injected into the blood of an experimental animal, causes the very evident increase in pancreatic secretion. The name "prosecretin" has been applied to this substance. The acid which normally causes the conversion of prosecretin into secretin is the hydrochloric acid of the gastric secretion; the acid solution escapes through the pylorus and enters the duodenum. If hydrochloric acid is to activate prosecretin, it must be in excess of that which is necessary to neutralize the alkaline secretions of the intestine. In gastric carcinoma in which there may be hypochlorhydria pancreatic hyposecretion may occur as a consequence of the lowered amount of HCl. If, however, gastric carcinoma involves retention due to pyloric stricture there may be a hypersecretion of HCl; and when this is released into the duodenum prosecretin is activated into secretin in an apparently normal manner.

The **reaction of intestinal juice** has been determined by several investigators. The variations are from *p*H 5.9 to 8.2. The average normal intestinal fluid is therefore slightly more acid than that of human blood, namely, 7.03, blood being 7.35.

The **amyloclastic function of pancreatic secretion; pancreatic amylase, or "amylopsin."** This is demonstrated by the following Exercise:

Exercise 11.—A glycerol extract must be used, acid destroying the enzyme. Cole's method: Place three test-tubes in your rack and add 10 mls. of 1 per cent. starch solution[2] to each; add also 8 drops of a 5 per cent. NaCl solution. Tube A is control; to B add 1 ml. of the pancreatic extract in glycerol solution; to C add 1 ml. filtered saliva. Then to each tube add 5 drops toluene and leave in the thermostat at 37° C. until the following period. Glucose is formed in Tube B and may be identified by the osazone.[3] Saliva, however, having no maltase in it accompanying the amylase, carries digestion to maltose. Identify these products by means of the osazones.

The Glucidases of the Pancreas.—The pancreatic enzyme corresponding to salivary amylase contains no maltase, but it is accompanied by a maltose-cleaving agent, which is a tissue enzyme and not

[1] Dale, H. H., National Institute for Medical Research, Hamstead, England.
[2] Page 147.
[3] Page 163.

an alimentary one. In the glycerol extract both enzymes occur, and hence the production of glucosazone in Exercise 11. When, however, pancreatic juice, obtained by means of a cannula or fistula directly from the pancreatic duct, is acidulated, maltose is hydrolyzed to two glucose molecules. This activation of maltase occurs in the acid condition of the duodenum much as secretin is produced from prosecretin. Amylase, apparently, does not have to be activated.

Diglucidases of the Intestine:

Exercise 12.—Prove the presence of lactase and sucrase or invertase as follows: Place separately in two small Erlenmeyer flasks 50 mls. of 2 per cent. solution of lactose and a similar amount of 2 per cent. solution of sucrose. As a control use other flasks containing 50 mls. of each solution in separate flasks. To each flask add 10 mls. of the glycerol extract of the intestine of the pig. Bring the control flasks to the boiling-point of water on a bath and then cool under the tap. To each of the flasks add 5 mls. toulene to avoid bacterial action. Place the flasks in the thermostat at 37° C. until the following period. Then make osazone tests for the identification of the products of hydrolysis of lactose and of sucrose, namely, galactose and glucose.[1]

Pancreatic Lipase.—For this work a special preparation of pancreatic extract should be made, details of which are given in the Appendix. The principle is to keep the extract alkaline.

Exercise 13.—Prepare the following tubes: A, control; 5 mls. of pure olive oil and one volume of the extract brought to the boiling-point of water for one minute and then cooled. Cover with toluene. B, prepared in the same manner, but left unboiled. Leave in a thermostat at 37° C. until the following period. Then titrate against decinormal alkali, with phenolphthalein as indicator, to determine the increase in acidity due to hydrolysis of the fat into glycerol and fatty acid. Calculate the amount of fat digested from the titration figure, supposing the oil to be composed wholly of trioleid.

Exercise 14.—*The Favorable Effect of Bile-salts on Pancreatic Lipase Action.*—Litmus milk preparation: Mix in a 100-ml. Florence flask one volume of whole milk and 0.1 volume of litmus solution.[2] To each of three test-tubes add 10 mls. of the mixture. To each tube add also 5 mls. of the special preparation of pancreatic extract used

[1] Fructose (levulose) does not give an osazone different from that of glucose. See page 165.

[2] Appendix.

in Exercise 13 just given. Bring the contents of Tube C (control) to the boiling-point. Cool under the tap and cover with toluene. Let Tube B remain as it is. To Tube A add 1 ml. 1 per cent. bile-salt solution.[1] Place the three tubes in the thermostat at 37° C. and leave for fifteen minutes. Examine the tubes and determine whether any change in color of the solution has occurred. Leave until some change is evident. The accelerating effect of the bile-salts should be made manifest.

The *explanation of the favorable effect of bile-salts in lipase action* is probably to be made in terms of physical rather than purely chemical factors. We have seen[2] how bile-salts lower the surface tension of water. They exert the same effect upon oil; the oil becomes emulsified, and since enzymes must be dissolved by fat[3] they do not exert their action in the interior of the fat globule. The smaller the fat particles, the greater the surface in proportion to the volume. Consequently enzymatic action occurs at the periphery of the fat globule. It is this emulsifying effect exerted by the bile when regurgitated into the stomach which permits the gastric lipase to become effective.[4]

BILE AND ITS BIOCHEMISTRY

Besides the bile-salts mentioned in the previous paragraph, bile consists of other substances:

Pigments.

Glucoprotid, mucin.

Cholesterol.

Phospholipid, lecithin.

Inorganic constituents, water, salts, etc.

It is a secretion of the hepatic cells, both Kupfer and epithelial cells. These cells likewise form glycogen and other substances. They represent the "laboratory" of the liver. The highly viscid, yellowish, or greenish secretion is collected from the cells and passes into the intercellular spaces (sinuses) which in their turn deliver the bile into intralobular ducts. The latter unite to form the bile-duct of the lobe of the liver in which they occur. The left and right hepatic ducts unite to form the common duct which opens in connection with the pancreatic duct (duct or canal of Wirsung) into the duodenum. The common bile-duct receives at its junction with the hepatic duct the

[1] Two mls. of a 10 per cent. solution of mammalian bile may be used. Commercial preparations of bile-salts (sodium taurocholate or glycocholate) are available.

[2] Page 116. [3] Page 118, note 1. [4] Page 117.

cystic duct, the interior of which bears a spiral valve. The cystic duct comes from the gall-bladder. The bile escapes into the intestine and in the lower portion of that organ it is absorbed to a greater or less extent into the blood. This makes up the so-called "enterohepatic circulation of bile." It is still an open question whether the bile pigments follow the circulation described; but it is probable that the bile-salts do.

From recent experiments of Rous and colleagues[1] an enterohepatic circulation occurs; for when green bile is fed, green and not yellow bile appears in the bile.

Secretin causes the increased secretion of the bile, but according to Downs[2] no other hormone has this effect; in fact, the flow of bile is decreased by epinephrin, testicular substance, ovarian tissue, pancreatic substance, and extracts of the thymus, spleen, and thyroid. That the secretion of bile is not merely a mechanical filtration is evidenced by the fact that epinephrin while increasing blood-pressure, decreases the flow of bile, and the biliary pressure is normally slightly greater than blood-pressure. Likewise, the osmotic pressure of the bile is slightly higher than that of the blood:

Depression of the freezing-point of blood	0.60° C.
Depression of the freezing-point of bile	0.61° C.

There is a parallelism between the excretion of bile and the excretion of urine. In both cases obstruction causes cessation of secretion.[3] A pressure of 350 mms. of water is sufficient to stop the flow of bile. This is about one-third the pressure required to cause the cessation of flow of the urine. Certain substances serve as cholagogues,[4] literally "drivers of the bile." Foremost among these substances are the intestinal purgatives, calomel (Hg_2Cl_2), aloës, rhubarb; these affect the flow, not the secretion of the bile. Bile-salts are supposed by many to be the only true cholagogues, but intubation examinations fail to substantiate that they are capable of exerting great effect.

[1] Brown, G. O., McMaster, P. D., and Rous, P. (Rockefeller Inst., N. Y.), Journ. Exp. Med., vol. 37, page 699, 1923.

[2] Downs, A. W., Physiologist, University of Alberta, Edmonton, Canada.

[3] Rous, P. (Rockefeller Institute, New York, N. Y.) has shown that obstruction of the bile ducts, in whole or in part, is followed by a secretion of "white bile" by the walls of the ducts into their cavities. The gall-bladder retains the characteristic dark bile, due to concentration. The clinical effects of obstruction of the gall tracts are of lesser importance compared to the effect of damage to the bile secreting portions of the liver. Jaundice of all forms is probably due to hepatic lesions rather than to duct occlusions.

[4] Greek *chole*, gall, and *agein*, from *ago*, to drive.

Protidtemns, like albumoses, peptones, etc., also serve in this capacity. Fats cause marked secretion of the gall-bladder bile. Magnesium sulphate introduced into the duodenum causes relaxation of the sphincter[1] of the common bile-duct, and bile is forced from the bladder at the next contraction *provided* the cystic duct is patent, or the tonicity of the gall-bladder is adequate, or, finally, the tonus of the duodoenal wall is sufficiently reduced.[2] Glucids exert a somewhat similar but less pronounced effect; however, it is more lasting. In

Fig. 156.—F. C. Koch, Professor and Director of the Department of Biochemistry, University of Chicago. Investigations concern enzymes; earlier work on secretin, lipids, etc.

fasting, bile is secreted to a lesser extent. Exercise has no effect upon biliary secretion. The amount of bile is lessened when the body is exposed to high temperatures doubtless owing to an increased excretion of water from the skin, thus lessening the water in the urine and other fluids like bile. There is a concentration of bile following exposure to high temperatures[3] corresponding to that in the urine

[1] Sphincter of Oddi on the papilla as the common duct enters the duodenum.

[2] See Matsuo, I., Jour. Amer. Med. Assoc., vol. 83, p. 1289, 1924.

[3] Castellani, A., Manual of Tropical Medicine, 3 ed., London, Baillière, Tindall and Cox, page 1906, 1919.

during hot weather. In the case of the bile there is a relatively increased amount of pigment in hot weather.

The **amount of bile secreted under normal conditions** is usually said to be about a liter per twenty-four hours. However, these figures are undoubtedly too low. By duodenal intubation as high as 4000 mls. may be removed in 24 hours. There may be some artificial stimulus by this method but scarcely to such an extent as that indicated by the larger amount. More is excreted in interdigestive stages (at night).[1] Since the chief functions of the gall-bladder are to concentrate bile from the liver and to deliver it to the duodenum when needed, it follows that the bile is more concentrated in the former. The bile cyst receives, say, a liter of bile from the secretory apparatus in the liver and this it concentrates to about 100 mls.[2] The gall-bladder is, then, not merely a temporary storage organ, but may cause a change in the consistency of the bile. The walls are 1 mm. thick when at rest and consist of mucosa, muscular, fibrous, and peritoneal zones. The mucosa is thrown into folds in which lie glands, lymph follicles, and channels which become associated as the neck of the gall-bladder is approached. Through these channels the water derived from the liver bile is passed, thus concentrating the bile. The average composition of bile follows (Lyon):

	Gall-bladder, per cent.	Common duct, per cent.
Water	86	97
Solids	14	3
Bile-salts	9	1
Mucin and pigments	3	0.5
Cholesterol	0.2	0.06–0.16
Lecithin and fat	1	0.9
Inorganic salts	0.8	0.7
Hydrogen ion-concentration, as *p*H	6.8	7.3–8

The Chemistry of Bile-salts.—The salts are composed of sodium and organic acids of which there are two: (1) Glycocholic acid and (2) taurocholic acid. These occur in equal quantities in human bile. Cholic acid[3] is chemically somewhat similar to cholesterol;[4] both have aromatic rings and alcohol radicles; but cholic acid lacks the terpene group of cholesterol, although it contains the characteristic aromatic

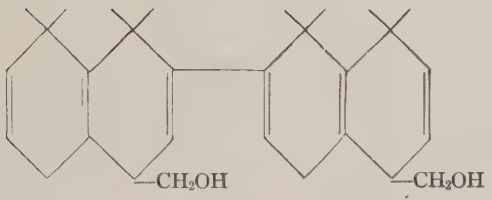
[1] Lyon, B. B., Vincent and Swalm, W. A., Journ. Amer. Med. Ass., vol. 85, p. 1541, 1925.

[2] Rous (see page 468) finds an average concentration of 7.1 times.

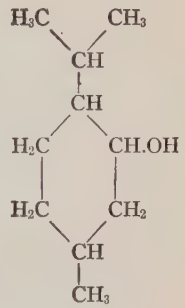
[3] Also called cholalic acid. Greek *chole*, gall. No relation exists with cholin.

[4] There is, however, no physiological reason for this relation.

groups represented by: , the skeleton of these compounds. Diagrammatically cholic acid may be represented thus:



Skeleton of cholic acid.



Menthol.[1]

The structural formula of an aromatic terpene, *menthol*, widely used in medicine, is given for comparison. The exact formula for cholic acid has not been determined, nor do we know that of a similar compound, choleïc acid, which is associated with cholic acid. The latter has a lower melting-point, greater solubility in ethanol, etc. Both acids conjugate with certain compounds like the amino-acid glycin (forming glycocholic acid and glycocholeïc acid, respectively), and the cystin derivative (forming taurocholic acid). These acids are not provided with the carboxyl-radicle which usually accompanies and is responsible for acidic qualities in organic compounds, but the acid qualities of glycocholic and taurocholic acids are due to the same properties that are responsible for the acid qualities of phenol (carbolic acid),[2] that is, the lowering of the basicity of the hydroxyl group by the acidity of the benzene ring, which is the nucleus in these compounds. Cholic acid is found in the urine of jaundiced subjects and normally in the intestine where it is formed by hydrolysis from bile acids. The rôle of conjugation products of cholic acid with glycin and with taurin seems to be, primarily, that of detoxication, of which we have encountered many examples; however, these products have other important secondary functions.

Glycocholic acid, $\begin{matrix}\text{COOH}\\|\\\text{CH}_2\text{NH}_2\text{C}_{24}\text{H}_{39}\text{O}_4,\end{matrix}$ occurs in the omnivorous or herbivorous human body, but in strictly carnivorous animals like dog, and man on a high protid diet, it is either present not at all or only in reduced amounts. It is distinguishable from taurocholic

[1] This substance is given the termination *-ol* because of the secondary alcohol in position No. 2. Otherwise it should be called menthene.

[2] Runge, who discovered phenol, termed it "carbon-oil" acid, or carbolic acid.

acid by being precipitated as the sodium salt by neutral lead acetate. It is highly toxic, slowing the heart by stimulating the vagus nerve and lowering blood-pressure. Glycocholic acid (or its salt) also causes lowering of the surface tension and consequently cytolysis, hemolysis, and other forms of cell destruction. The absorption of bile-salts is productive of hematogenous jaundice, the destruction of liver tissue being ascribed to this agent. At one time it was popular as an antiseptic for the urinary tract, but it was found that the effective dose incurred danger from cytolytic effects upon the cell and erythrocytes. Glycocholic acid is an effective cholagogue, causing increased secretion of the bile.

Taurocholic acid, $\begin{matrix} CH_2.HSO_3 \\ | \\ CH_2NH \end{matrix} C_{24}H_{39}O_4$, represents a similar conjugation to that of glycocholic acid. We have discussed the chemistry of taurin on page 253 as a derivative of cystin. It is distinguishable from glycocholic acid by being precipitated by "basic" lead acetate, but not by the normal acetate. Moreover, it is readily soluble in water, whereas glycocholic acid is refractory toward water. It is typically absent from the blood of some animals, and the low sulphur content of human bile[1] indicates that taurocholic acid is present in small amounts. The relative amounts of the two typical bile acids in human bile is a matter of diet. There is, apparently, a limit to the amount of taurin in the body available for making taurocholic acid, whereas glycin is available at all times; hence glycocholic acid may be present when taurocholic acid is either absent or present only in small amounts. The source of cholic acid is probably the sterols which include cholesterol of the bile and blood, and dietary sterols, like phytosterol. Consequently, herbivores have more bile acids than do carnivores. The relation between cystin, taurin and taurocholic acid is well shown by feeding experiments. Cystin, when fed alone, appears in the urine as sulphuric acid, CO_2 and H_2O; if sodium cholate is administered at the same time the taurocholic acid of the bile is increased and the sulphuric acid of the urine is correspondingly decreased. Moreover, in cystinuria[2] there is a failure of the synthetic powers of the body to cause this conjugation. If sodium cholate is ingested, the maximal quantity of sulphuric acid will be found in the urine, but no increase in taurocholic acid in the bile.

[1] Sulphur content of human bile salts, 0.1 per cent. dry weight; of steer bile-salts, 3.5 per cent.; of sheep bile-salts, 5.71 per cent. (Hammarsten.)

[2] Page 252.

Bile-salts are Conjugated in the Liver.— In cirrhosis of the liver there is acholia, or suppression of bile formation, and cystin appears in large amounts due to damage to the synthesizing apparatus in these cells. Although, as we have stated, food determines the relative proportion of the two acids, yet the fact that both are secreted during starvation shows that they are partly at least of endogenous nature.

Summary of the Economy of Bile-salts.—(1) They are conjugated cholesterol-like substances (cholic acid) with (*a*) the amino-acid, glycin, or glycocoll, forming glycocholic acid; or with (*b*) the amino-acid derivative, taurin (from cystin), forming taurocholic acid. There is no evidence, other than chemical similarity, that the bile-salts come from cholesterol.

(2) In the bile they exist as sodium salts of these acids.

(3) They are highly toxic,[1] having (*a*) cytolytic action; (*b*) cardio-depressor action; (*c*) vaso-dilating action (small amount) or constricting action (larger amounts); (*d*) reduce nervous irritability, causing coma in larger amounts.

(4) They are of both endogenous and exogenous origin.

(5) They aid in the (*a*) digestion and (*b*) absorption of fats; they cause the emulsification of fats and they aid in the passage of the lipid-temns through the intestinal membrane forming loose union with the fatty acids.

(6) They are cholagogues, the only substances known to cause much increased secretion of bile. It must be admitted, however, that duodenal drainage observations do not bear out this sweeping statement.

(7) They hold cholesterol in solution in the body fluids.

Qualitative Tests for the Presence of Bile-salts.—We have given elsewhere[2] Hay's test for bile-salts based upon the property of lowering surface tension. It is repeated here for completeness:

Hay's Test for Bile-salts.—Place some of the fluid suspected of containing bile-salts in a test-tube and hold under cold water to reduce the temperature, which enhances the effect. Now sprinkle as much flowers-of-sulphur (resublimed) over the surface as will adhere to a knife-point. The test is positive for bile-salts if the sulphur passes through the surface and sifts to the bottom of the tube. Any fluid having a surface tension lower than 60 dynes per square centimeter

[1] In toxic cases, we note the increase in the proportion of bile-salts to bile.

[2] Page 116.

of surface gives the test; consequently, alcohol, chloroform, etc., used in preserving urine, give a falsely positive test.

The above test is a physical chemical one, the explanation of which is given on page 115. The test must be used with caution, since substances other than bile-salts will cause the same reaction. Preservatives, substances used in treatment of respiratory diseases, etc., may exert this influence and give a falsely positive test.

The following test depends upon the same chemical reaction that we have noted in Molisch's Reaction and the Selivanoff Test for glucids, Hopkins' thiophen reaction for lactic acid, etc., namely, a condensation of an aldehyde with an aromatic substance. The aldehyde, furfural, in the present case is derived from sucrose by hydrolysis with H_2SO_4; the aromatic substance is the bile-salt.

Pettenkofer's[1] *Test for Bile-salts.*—Place 5 mls. of the solution to be tested in a test-tube and add a few grains of common table sugar, sucrose. Shake the solution until the crystals are dissolved, warming if necessary. Cool under the cold-water tap. Then layer with concentrated sulphuric acid; the acid underlies the solution. Carefully mix the contents of the tube by inclining it one way, then the other. Note the appearance of color at the junction. Similar color reactions are given by glucoprotids, like mucin, and since the bile contains such substances care in reporting a positive must be observed. The urine, likewise, contains aromatic substances, pigments, which imitate bile in giving these reactions. Protid itself gives a positive bile-salt reaction; so do protidtemns like pepton.[2] The Pettenkofer Test works best with pure solutions, protid free.

Bile Pigments.—These are derivatives of the red coloring-matter of the blood, hemoglobin, which is cleaved into (1) an iron-bearing part, hematin, and (2) an iron-free part, globin.[3] Hematin becomes changed in the liver and elsewhere to a variety of pigments.[4] They

[1] Pettenkofer, M. von, died 1901. Chemist in the Physiological Institute, Munich, Bavaria, Germany.

[2] This property is taken advantage of in Oliver's (Thomas Oliver, contemporary English physician) Test for bile-salts, but in this test, in place of the color reaction depending upon the presence of aldehyde-rendering substance, it depends upon a chemical union between bile-salts and protid, or pepton. For Oliver's test see Dictionary (cited on page 463, note 1) page 1174. [3] Page 324.

[4] Some bile pigment may arise in the body other than in the liver, and substances other than hemoglobin may give rise to these substances. However, see Rous, Peyton, Jour. Exp. Med., vol. 37, p. 421, 1923. Also Mann, F. C., Bollman, J. L., and Magath, T. B., Amer. Jour. Physiol., vol. 69, p. 393, 1924. See also: Mann, F. C. The site of formation and source of bilirubin. Archives of Path. and Lab. Med. (Amer. Med. Assoc.), vol. 2, page 516, 1926.

resemble hemoglobin in being weak acids and they form salts like sodium, potassium, and calcium, the first and last being especially important from the physiological standpoint. In addition to the chemical changes which these hemoglobin derivatives undergo in the liver, other products are formed by bacteria in the intestine, and while these compounds are excreted via the feces, some are absorbed into the blood. Chlorophyl, closely allied to hemoglobin, shares a like fate, but from chlorophyl arises a special pigment, chlorhematin or bilipurpurin. The excretion of bile pigments is endogenous, being constant under ordinary conditions, averaging 7.5 mgs. per kilo of body weight.

The *origin of bile from extrahepatic sources* has been demonstrated by Rich,[1] who finds that phagocytic cells, the so-called "clasmatocytes,"[2] ingesting erythrocytes in living cultures, may be seen to form bile pigments. Moreover, there is evidence that reticulo-endothelium[3] can perform similar pigment syntheses. A third reason for believing that bile pigments can be formed outside the liver is that when the liver is entirely cut out of the circulation of the pigment still forms.[4] Venous blood of the spleen and bone-marrow is richer in bilirubin than arterial, showing that this pigment is produced in these organs.

Chemistry of Bile Pigments.—Like hemoglobin, the bile pigments are pyrrol compounds. We have already discussed the chemistry of hemoglobin[5] and traced its reduction as far as the iron-free compound, hematoporphyrin. This in turn becomes reduced to hemoporphyrin, the structural formula of which is given on page 383. From hemoporphyrin is derived a pigment such as occurs in old blood-clots known as hemosiderin. This is identical with one of the bile pigments, bilirubin[6] and we shall use the latter name. The structural formula of bilirubin as given by Hans Fischer[7] is as follows; compare it with that of hemoporphyrin on page 383.

[1] Rich, A. R. (Johns Hopkins University, Baltimore, Md.). See Johns Hopkins Hospital Bulletin, vol. 35, p. 415, 1924. Also Phys. Revs., vol. 5, p. 182, 1925.

[2] Greek *klasma*, fragment, and *kytos*, cell.

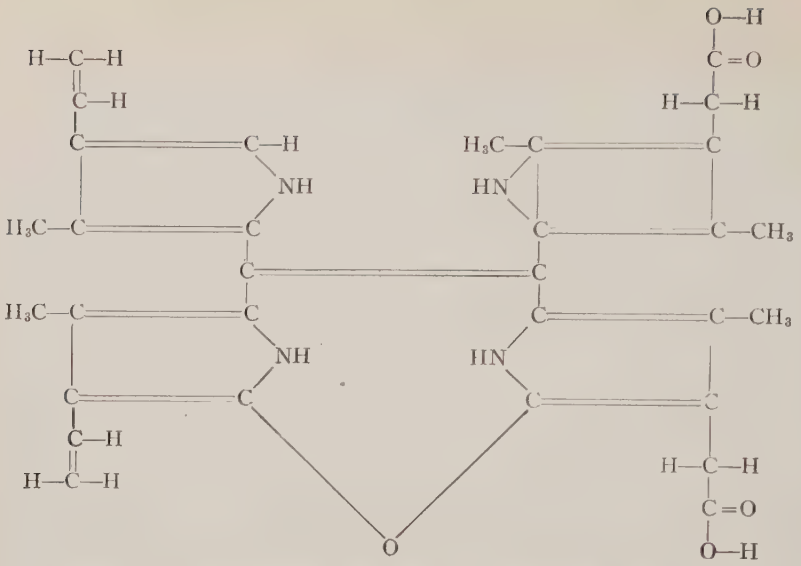
[3] Page 115.

[4] Rich, A. R., Bull. Johns Hopkins Hosp., vol. 36, p. 233, 1925.

[5] Page 382.

[6] From the Latin, *bilis*, bile, and *ruber*, red; literally "red-bile." The color is actually brick red with much brown in it. See page 385.

[7] Fischer, Hans, German physician, Professor of Internal Medicine, University of Munich, Bavaria, Germany.



Bilirubin is characteristc of the bile of carnivores and of man on a high protid diet. It occurs in the hepatic duct in all vertebrated animals, but may become oxidized in the cyst to form biliverdin. It occurs in gall-stones as the calcium salt, calcium bilirubinate. It gives the stomach contents a yellowish tinge and may even color the blood-serum[1] by its presence. Similarly, the yellow color of the feces when distinctly golden yellow[2] is due to this pigment. This indicates that diarrheal condition where rapid movement of the intestinal contents carry bilirubin below the ascending colon. Normally, bilirubin is not found below this organ. In the transverse and descending colon and in the rectum, hydrobilirubin[3] is the normal pigment; this is due to the action of bacteria. Further action, involving loss of some of the nitrogen of the molecule, produces urobilin ("stercobilin"). It is probable that hydrobilirubin is not a single compound, but consists of two parts, one being identical with the so-called urobilinogen[4] of the urine and the other yielding certain acids, like hematinic acid, an oxidation product of hematin.

[1] Such blood shows also a foam formation due to the presence of mucin (see Udranszky's Test, page 116).

[2] A bright lemon yellow is imparted by the substance santonin; see Chapter XVII.

[3] Responsible for the black color of the stools.

[4] Since this was found by Hans Fischer to form about one-half the whole substance, hydrobilirubin, it was called "hemibilirubin," but it was then found to be identical with the pigment of the urine, urobilinogen, the mother substance of the urobilin of the urine and feces, which increases in certain liver involvements like cirrhosis, infections, lead-poisoning, in disease of the adrenals, etc. For the test of this urinary pigment see Chapter XV.

The *oxidation of bilirubin* produces the second bile pigment of importance, biliverdin.[1] This property is utilized in several tests for bile-pigments, including that of Huppert,[2] Fouchet,[3] etc. Biliverdin is characteristic of the bile of herbivores and of man on a high vegetable diet.[4] Bile regurgitated into the stomach is typically green owing to the oxidation of the bilirubin to biliverdin. Likewise, vomitus is tinged green by biliverdin.

As we shall see in a practical way (page 479), wherever bile may form, whether in the liver or extrahepatically, the manner of transmission from the blood to the bile-system varies under different pathological conditions. If bilirubin follows its normal course, it is formed in the reticulo-endothelium in various parts of the body, but especially in the marrow of the long bones. Then it is passed into the bile ducts from the blood *via* the polygonal hepatic cells. If, however, hemolysis of erythrocytes occurs as in hemolytic jaundice, then the Kupfer cells transmit the bilirubin they make as a part of the reticulo-endothelial system, directly into the blood. If there is an obstruction of the biliary system, then the bilirubin is transmitted through the polygonal cells into the blood. The two types of van den Bergh reaction (page 479) depend upon the behavior of bilirubin; obstructive jaundice gives an immediate response, hemolytic gives a delayed reaction. By spectro-photometric observations, Mann (page 474) is unable to find any difference between gall bladder bile pigment, pigment of obstructive jaundice, pigment of dehepatized pigment, bone-marrow pigment and the pigment found in blood after introducing hemoglobin into the blood stream.

Summary for Bile Pigments.—(1) They are usually derived from hemoglobin in the liver, although they may be formed in other locations and from other substances.

(2) The chief pigment is bilirubin, which is converted into biliverdin after it leaves the liver. In herbivores, the preponderance of biliverdin is doubtless due to the retention of biliary secretions in the gall-bladder and in the intestines, which are especially long and involved in such animals.

[1] From the Latin *bilis*, gall, and *viridis*, growing; that is green; literally, green bile.

[2] Huppert, H., Bohemian physician, died 1904. See page 478.

[3] Fouchet, H., French clinician; see Comptes rendus, vol. 80, p. 826, 1917.

[4] However, a glucid diet causes increased excretion of the bile and this may cause a rapid removal of liver bile through the common duct. Liver bile is preponderatingly colored with bilirubin.

(3) Bacteria affect the bile pigments, converting them into other substances which are absorbed into the blood, and these are excreted through the urine.

(4) Our present knowledge of bile, and especially of the pigments, gives us no reason to believe that the excretion of bile pigments is a process concerned with anything other than excretion; that is, bile is not excreted for any purpose other than to rid the body of this material. The "circulation of bile" concerns only the bile salts.

(5) Clinically, the bile gives evidence of lesions of the liver, retention, etc. Thus, the white stools of the child may be due to (*a*) diarrhea, in which case bilirubin gives the diluted yellow or clay color;[1] or (*b*) to retention of the feces, in which case bilirubin is changed, by bacteria, into colorless pigment, leucohydrobilirubin.

Qualitative Tests for Bile Pigments:

Huppert's[2] *Test.*—Principle: Bile pigments are precipitated as calcium salts and bilirubin is oxidized to biliverdin, which imparts a green color. Procedure: Boil a test-tube of the bile solution[3] and add 2 drops of a saturated solution of magnesium sulphate;[4] then add 10 per cent. solution of barium chlorid, drop by drop, as long as a precipitate forms, after the solution is brought to the boil. Let stand to permit the precipitate to settle; or centrifuge the solution. Pour off and discard the supernatant fluid. To the residue add 5 mls. of 95 per cent. ethanol and acidulate with 2 drops of concentrated sulphuric acid and a similar amount of 5 per cent. solution potassium chlorate, $KClO_3$. Boil. Let cool and the precipitate settle. The alcoholic layer above will then turn green. The color may be intensified by shaking out the pigment in the decanted alcohol layer with chloroform, adding one volume of water, and then allowing the chloroform layer to separate. The resultant color will be bluer.

Gmelin's[5] *Test.*—Principle: The usual bile pigment, bilirubin, is oxidized to a variety of substances, each having a characteristic color. Among these are biliverdin, etc. Procedure: Filter the solu-

[1] The clay color may be due also to a high fat content in the stool.

[2] Page 477.

[3] If pure bile is used, dilute it with 10 volumes of distilled water before making the test.

[4] Equal parts by weight of $MgSO_4$ and H_2O mixed at room temperature. During the mixing the temperature of the solution will fall to about 10° C. or lower, at which temperature the solution will be saturated; if the solution is used at 20° C., then add 75 grams more to the solution, mix, and apply to the test as directed in the text.

[5] Gmelin, L., German physiologist, died 1853.

tion suspected of containing bile through ordinary filter-paper in a funnel and then bring the paper, unfolded, into the fumes of a bottle of fuming nitric acid;[1] note the play of colors.

Confirming Method: Place 2 or 3 drops of the solution being analyzed on a porcelain plate or in an evaporating dish. Let the solution concentrate, or warm it carefully. Add a drop of fuming nitric acid to the spot, and note the play of colors.

Confirming Method: Overlay 5 mls. of fuming nitric acid with one volume of the solution being tested. Incline the tube so that the two liquids become slightly mixed. Note the colors.

Fouchet's Test.—The reagent: Make the following solution fresh each time: To 20 mls. distilled water add 2 mls. $FeCl_3$ 10% solution, and 5 grams of crystals of trichloracetic acid. Mix. The procedure: Place 3 drops of blood serum which shows no hemoglobin on a white surface (spot-plate) and add one volume (3 drops) of the reagent. The serum protids are precipitated by the CCl_3COOH as a white coagulum. If bilirubin is present, the ferric chlorid oxidizes the pigment to greenish-blue biliverdin. Examine after about 20 minutes. Is there a change in intensity?

The van den Bergh[2] *Reaction.*—Principle:[3] This test distinguishes (I) bilirubin that is derived wholly from hepatic cells that stand between the blood and bile apparatus (polygonal cells); (II) bilirubin derived wholly from the reticulo-endothelium (page 475); and (III) bilirubin from both sources. Reagents: Ehrlich's di-azonium solution as follows:

1. Diazo-solution:
   Solution A.
      Sulphanilic acid—1.0 gram.
      Hydrochloric acid, conc. 15.0 mls.
      Water, distilled—1000.0 mls.
   Solution B.
      Sodium nitrite, stick—0.50 g.
      Water distilled—100.0 mls.
2. Ethanol, 99.6 per cent. ("absolute").

Note: The substance formed by the interaction of sulphanilic acid and sodium nitrite is a diazonium compound that azotizes bilirubin to form a purplish azo-bilirubin.

[1] Appendix.

[2] Van den Bergh, H. (Dutch pathologist) Die Gallen-Farbstoff im Blut. Leiden, Holland; 1918.

[3] See McNee, J. W. Quart. J. Medicine, vol. 16, page 390, 1923.

Procedure.—Using only serum that has been carefully pipetted from about 10 mls. blood after clotting, transfer 1.0 ml. into a small testube and add 0.25 ml. of (1) mixed in the proportion of 25 mls. of A to 0.75 ml. of B, immediately before use. The result is, if positive:

(1) Immediate: a bluish violet color in 10–30 secs. Polygonal cell bilirubin.

(2) Delayed; reddish color, becoming violet, beginning in 15 minutes. Kupfer cell bile.

(3) Diphasic; the reddish color comes at once but takes much longer to become violet.

In a negative test of 1 and 2, try the *indirect* reaction: Add to 1.0 ml. of fresh serum in a 15 ml, centrifuge tube, 2.0 mls. of ethanol "abs." Mix. Centrifuge. Pipette 1.0 ml. of the clear supernatant fluid into a small test-tube, add 0.5 ml. ethanol as before and 0.25 ml. reagent. If positive, an immediate appearance of a violet color is observed.

Biliary Mucin.—This substance is a glucoprotid which adds to the viscosity of the bile. It may be precipitated by a method similar to that used for saliva;[1] that is, by adding acetic acid. Such a precipitate will contain not only mucin but also nucleo-albumin,[2] a phosphoprotid resembling ovovitellin and casein. The true mucin is probably secreted by the mucosa of the gall-bladder and the ducts. Its function may be that of lubricating the bladder bile, which may become very thick and tenacious, or indeed it may serve to aid in the passage of biliary concretions (gall-stones). The viscosity of mucin is responsible for Udranszky's test (page 116).

Phospholipids occur in the bile, but definite members of the group have not been identified. It is probable that lecithin is one of them. The function and origin of these phospholipids is still unknown.

Inorganic Substances.—Nearly 90 per cent. of gall-bladder bile and 97 per cent. of liver bile are water. We have called attention to the function of the gall-bladder of disposing of the water of liver bile, which concentrates the bladder bile. There is a wide-spread misconception of the reaction of bile as being highly alkaline. Bile is normally neutral or alkaline, and it may become slightly acid. Titration of bile to determine its reaction gives erroneous results, for it determines the total hydrogen-ion concentration possible, and not that

[1] Page 320.

[2] Not nucleoprotid, as books frequently state, for there is no glucid in its hydrolysis products as there is in the case of the nucleoprotids.

which exists at the time and under the conditions prevailing when the determination is made.[1] Moreover, like all body fluids, bile is highly buffered[2] and, consequently, changes in hydrion concentration occur only when a considerable addition of acid or base is made. The statement is frequently made that the bile is alkaline and that its alkalinity serves to neutralize the acid in the stomach by regurgitation; or the neutralization may occur when the acid contents pass into the duodenum. The alkalinity of the bile alone is seldom adequate to do this. It is possible that buffers of the bile (due to alkali united to the bile-salts[3]) may be adequate to take care of much acid. Iron is present in bile to a small extent, 0.01 per cent. Traces of copper and of zinc have been found, the origin of which is unknown.

Cholesterol and the Formation of Biliary Calculi (Gall-stones).— Cholesterol was first found in gall-stones and given the name "solid bile."[4] It forms about 0.1 per cent. of the bile in the liver and about 0.2 per cent. of that in the gall-bladder. The latter is approximately the concentration of cholesterol in the blood.

The *origin of cholesterol* of bile is not known, by there are indications that it may be derived from the cholesterol of the blood and of the foods. In the first place, there is little evidence that cholesterol is synthesized in the human body[5] and, therefore, we should expect phytosterol and other sterols admitted as food to supply the cholesterol of the blood. This expectation has been substantiated by Gardner,[6] who finds that of the total phytosterol taken in with the foods, only a part is absorbed, the remainder being reduced by bacterial action in the intestine to koprosterol and β-cholestanol, a derivative of phytosterol.[7] Second, cholesterol is found in the periphery of the erythrocytes and also free and in the form of cholesterol esters in the blood. Since many substances occurring in the bile

[1] See momentary acidity, page 87.

[2] Page 81.

[3] Sodions and phosphions united into the substances NaCl, KCl, $CaCl_2$, etc. Protids, like phosphoprotid and mucin, also serve as buffers.

[4] Page 214.

[5] Gardner, J. A. (University of London), and Fox, F. W. (St. George's Hospital Medical School, London), have reported that there is about 0.3 g. of cholesterol which must be accounted for in the average subject, per day, by synthesis in the body. See Proc. Roy. Soc. London, Series B, vol. 92, p. 358, 1921. Also see Thannhauser, S., Deutsch. Arch. f. klin. Med., vol. 141, p. 290, 1923, who finds no evidence of cholesterol synthesis. Lastly, Channon (H. J. University College, London) finds evidence of cholesterol synthesis in experimental animals (Biochem. Jour., vol. 19, p. 424, 1925).

[6] Gardner, J. A., Biochem. Jour., vol. 15, p. 244, 1921.

[7] Beta-cholestanol is obtained by artificial reduction in the test-tube with hydrogen in the presence of a catalyzer.

are derived from blood (pigments, etc.), it is fair to assume that cholesterol is derived in a similar manner.

The *function of cholesterol* in the bile has not yet been determined. However, the close chemical relationship existing between cholesterol and the cholic acid of bile salts would suggest that these salts have their origin in cholesterol. The fact that the cholesterol of the gall-bladder is more concentrated than any other ingredient of the bile in the bladder has led to two assumptions: (1) Cholesterol is secreted by the walls of the gall-bladder, and (2) the greater concentration is due to a reversal of reaction, leading to the formation of cholesterol from bile-salts. Studies of cholesterol in the intestine require differentiation between this substance and other sterols, such as phytosterols. This is so difficult to accomplish that the fate of cholesterol in the body still remains unknown. Certainly, much of it is lost in the stool as koprosterol, the characteristic sterol of the feces.

Cholesterol is found in many organs in pathological states. Wherever absorption of products of metabolism is interfered with, cholesterol is found, as, for instance, in atheromatous[1] arteries, hydrocele[2] fluid, etc. It does not undergo autolysis and so persists when other substances are destroyed. Therefore, cholesterol may possibly be a by-product, excreted by way of the bile. Bloor has shown that some of the fat of the blood is transported as cholesterol esters of the fatty acids and the alcohol, cholesterol. It may be that when this purpose is accomplished, cholesterol becomes a waste-product.

Gall-stones (biliary calculi) are formed in the gall-bladder under conditions not completely understood. Various theories regarding their origin have been presented. In this connection, certain facts should be reviewed: (1) cholesterol has so slow a solubility in water that it may be considered partially insoluble. (2) The principal agent maintaining the cholesterol in solution in the body fluids are the bile-salts, which vary in concentration in different persons (3) Additional agents holding cholesterol in solution are the lipids, neutral fats, phospholipids, etc. (4) Bile in the gall-bladder is in a static condition, which, in other fluids, is conducive to the deposition of cholesterol. The bile is formed under low pressure and its viscid nature accentuates the slowness with which it passes from the liver, gall-bladder, and common duct. (5) Cholesterol is precipitated by colloids of electropositive nature, as are all other biliary components (pigments, etc.) added to the bile during a catarrhal affection of the ducts or blad-

[1] Greek *athere*, oatmeal porridge; hence softening of the walls of the vessels.
[2] Greek *hydor*, water, and *kele*, a lump (tumor).

der mucosa (mucin and phosphoprotid). All of these facts and principles point to the possible origin of gall-stones from some lesion or pathological condition of the biliary apparatus. On the other hand, cholesterol is increased in the blood in certain diseases, notably infections of the biliary tract due to the migration of intestinal bacteria (typhoid, influenza, coli, etc.) into the biliary apparatus. In pregnancy there is an increase of cholesterol in the blood.[1] Certain races are more subject to gall-stones than others and in such instances, hypercholesteremia, or increased cholesterol in the blood, is present. In such races there is usually a higher cholesterol content in the food. We cannot here go into the various theories regarding the origin of gall-stones, but the following statements are based upon recent researches in this field:[2]

Types of Gall-stones.—Gall-stones are of two kinds (Fig. 157): (A) *Of non-inflammatory origin*, and (B) of *inflammatory origin*. There are two characteristic forms of stones belonging to the first group: pure cholesterol stones, the planes of crystalline cleavage being radial; and pigmented calcium stones. The former have the following characteristics:

(1) Composed of nearly pure cholesterol, intermixed with small amounts of protid and calcium.

(2) When the stone is sectioned, or broken, the structure is coarsely crystalline; there are no lamellæ or plates which are characteristic of other forms of stones.

(3) They have been called "cholesterol solitaires" because of their occurrence singly.

(4) They occur chiefly in persons who have never shown any indication of gall-stone trouble.

(5) They occur in persons of any age, even in children. They are found in women after pregnancy has begun.

The origin of this form of stone is attributable to increased cholesterol in the blood. This increase may be connected with fat metabolism in the manner suggested above.[3] These latter statements are not accepted by Naunyn,[4] but Aschoff believes that the pure cholesterol stones are responsible for about one-third of all the varieties of

[1] Chapter XVI.

[2] For an account of modern theories of gall-stone formation see the following: Aschoff, L., Lectures on Pathology (delivered in the United States, 1924). New York, Paul B. Hoeber Inc., 1924, Chapter IX, The Origin of Gall-stones.

[3] Page 851.

[4] Naunyn, F. (German clinician, formerly of Strasbourg), see the book, Die Entstehung und die Aufbau des Gallenstein, Jena, Gustav Fischer, 1923.

gall-stones. They are either pure cholesterol stones as such, or pure cholesterol stones combined with other substances to form the so-called "combination stones," which are the second kind (B) of gall-stones referred to on page 483.

The *origin* of this second group of stones is explained by Aschoff as follows: Hypercholesteremia leads to the formation of pure cholesterol stones. These fill the lumen of the gall-bladder and lead to

FIG. 157.—Different types of gall-stones: Above, faceted cholesterol-pigment-calcium stones. Below: Left, cylindrical cholesterol pigment stone; right, true cholesterol stone, *i.e.*, combination stone with cholesterol nucleus.

inflammation of the neck of this organ. Protid and bile pigment (bilirubin) from the bile are intermingled with the pus and débris from the inflammation. Calcium is one of the important constituents, especially of the cortex of the stone; the calcium is derived from the mucous membrane of the gall-bladder. Such combination stones occur in numbers, but there is only one true cholesterol stone in the group, the others, always smaller, and lacking the radiating crystalline nucleus, but consisting of the same material that makes up the cortex of the true stones. These "false" stones are always faceted as shown in Fig. 157. On sectioning, one finds that the layers are not arranged radially, but in lamellæ, or plates.

A stone, characteristic of the gall-bladder, but found also in the hepatic duct and in the smaller bile-ducts, is the *pure pigment stone.*

It occurs in large numbers in sizes from that of a grain of rice to that of a pea. They are called frequently "mulberry stones" owing to the resemblance to that form of fruit. In consistency they are firm, brittle, and difficult to cut with a knife. Sometimes they are found in the form of a disintegrated mass, or coarse black sand. They are formed as small concretions, having no nucleus or cortex.

The *pure cholesterol stones* are formed in a medium poor in protid, while the cholesterol-pigment-culcium stone arises in a medium rich in protid.

These two types are supplemented by a third group of stones arising through the stasis of the contents of the bile bladder. They are the "*earthy*" *stones*, consisting of calcium and bilirubin. They are lamellated, less dense than other stones, and are laid down around some nucleus, chiefly a pure cholesterol stone. They make a combination stone of the second order.

The Analysis of Biliary Calculi.—For clinical purposes it is frequently desirable to know the chemical and physical composition of gall-stones. We have just discussed the various kinds. They are presented here in four groups:

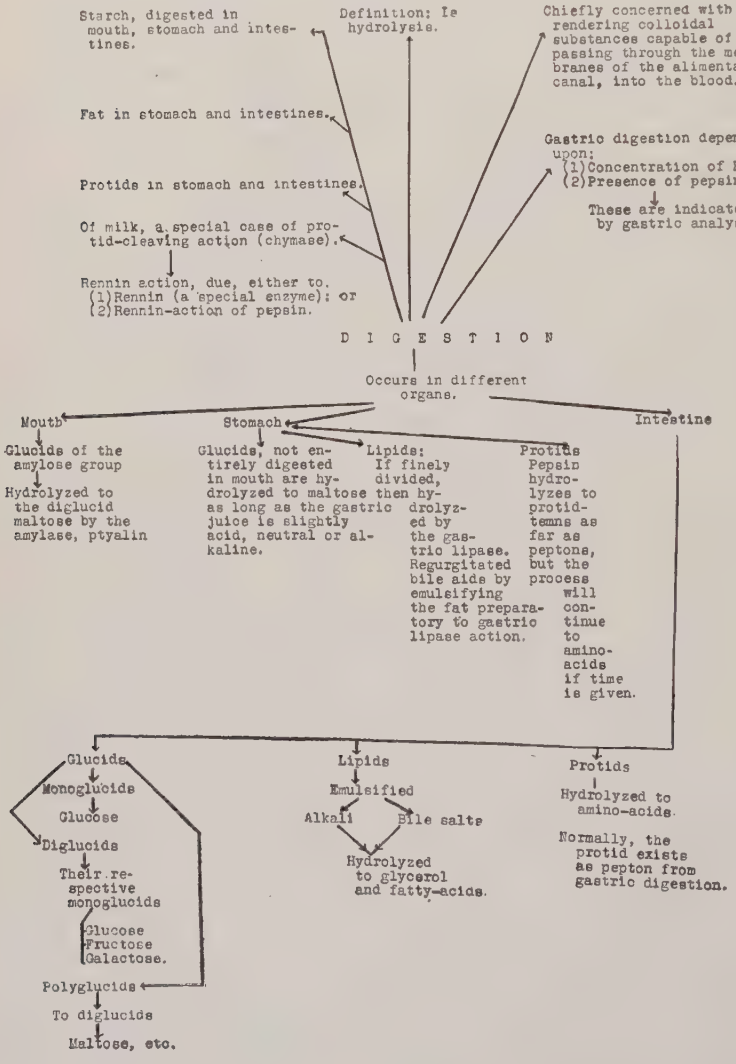
(1) *Pure Cholesterol Stones.*—By means of a small hammer fracture the stone and observe the layers of cholesterol in the form of "interwoven tree-trunks," not in plates. Test for protid is not pronounced and may be negative. Test for calcium is generally positive, but not pronounced. They show no facets, because they are formed singly.

(2) *Combination Stones.*—These stones may be "true" or "false." The true stones have a cholesterol nucleus, with bilirubin and calcium in the cortex. The false stones are found in the same group as the true stone (of which there is only one in the group, but they lack the cholesterol nucleus. They are faceted, owing to being packed into small space. Scrapings from the external surface, treated with concentrated HCl, will demonstrate the presence of calcium carbonate by the evolution of CO_2. These stones are lighter than water and therefore float when thrown upon the surface of water in a beaker.

(3) *Pure Pigment Stones.*—Of small size and usually of "mulberry" shape and appearance. They are not easily crushed. The fractured stone shows no zones of nucleus, cortex, etc. There is no effervescence when HCl is applied to the external surface. Qualitative tests for iron and for copper are positive. Test for copper: Crush a stone and treat it with 1 drop of concentrated hydrochlorid acid. Dilute with distilled water to make about 2 mls of solution. Add excess desk reagent concentrated ammonium hydroxid. A blue color of cupric hydroxid indicates a positive test. These stones sink in water.

(4) *Lamellated Calcium and Bilirubin Stones.*—They are less dense than the mulberry stones (3). Test for phosphate[1] is strongly positive. Bile pigments may be identified in gall-stones by shaking up the ground stone with chloroform, and filtering. Bilirubin is demonstrated on the filtrate by adding one volume of distilled water, 2 drops of concentrated H_2SO_4, and 2 drops of 5 per cent. solution of potassium chlorate. Heat the solution. Let cool and note the green color of the choloroform layer.

GRAPHIC SUMMARY


Starch, digested in mouth, stomach and intestines.
Definition: Is hydrolysis.
Chiefly concerned with rendering colloidal substances capable of passing through the membranes of the alimentary canal, into the blood.
Fat in stomach and intestines.
Gastric digestion depends upon: (1)Concentration of HCl. (2)Presence of pepsin.
These are indicated by gastric analysis.
Protids in stomach and intestines.
Of milk, a special case of protid-cleaving action (chymase).
Rennin action, due, either to. (1)Rennin (a special enzyme); or (2)Rennin-action of pepsin.
D I G E S T I O N
Occurs in different organs.
Mouth
Stomach
Intestine
Glucids of the amylose group
Hydrolyzed to the diglucid maltose by the amylase, ptyalin
Glucids, not entirely digested in mouth are hydrolyzed to maltose as long as the gastric juice is slightly acid, neutral or alkaline.
Lipids: If finely divided, then hydrolyzed by the gastric lipase. Regurgitated bile aids by emulsifying the fat preparatory to gastric lipase action.
Protids Pepsin hydrolyzes to protidtemns as far as peptone, but the process will continue to amino-acids if time is given.
Glucids
Monoglucids
Glucose
Diglucids
Their respective monoglucids
Glucose Fructose Galactose.
Polyglucids
To diglucids
Maltose, etc.
Lipids
Emulsified
Alkali
Bile salts
Hydrolyzed to glycerol and fatty-acids.
Protids
Hydrolyzed to amino-acids.
Normally, the protid exists as pepton from gastric digestion.

[1] Page 360.

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CHAPTER X

THE ABSORPTION OF FOODS

"The extreme assumption that the laws of physics and chemistry are inadequate to explain the causation of vital phenomena is, of course, not justifiable, for it postulates that we fully comprehend now all the laws of the physical world."—*Macallum*,[1] "Surface Tension and Vital Phenomena."

BIOLOGICALLY, the alimentary canal is an inclusion from the outside—an incorporation, as it were, of the environment. Functionally, it is much the same. Foods enter the canal through the mouth; yet these foods cannot be utilized unless they happen to be in a suitable physical or chemical state for absorption. Digestion constitutes the series of processes which so alter the foods ingested that they may be assimilated.

ABSORPTION

The process whereby the foods (whether already in a state fitted for assimilation or rendered so by the process of digestion) *are assimilated by the organism is called absorption.*[2] This involves a passage of the food substances through membranes which form the walls of the alimentary canal and through the circulatory system which carries them to the different parts of the body.

In What Portions of the Canal Does Absorption Take Place?—The food remains in the mouth for so short a time that we should scarcely expect this organ to be solely absorptive in function. The same may be said of the esophagus. The stomach is different, however, for the food remains there for a period varying from a few minutes, in the case of pure substances like glucose, to over four hours in the case of protids. Nevertheless, the structure of the walls of the stomach does not indicate an absorptive function. With regard to the absorption of water in the stomach, it has been known for many years that patients suffering from pyloric stenosis become very thirsty even when copious amounts of water are given by mouth; the water is not absorbed into the system. On the contrary, it passes through

[1] Page 24; also Fig. 172.

[2] The term "resorption" has been used; since the prefix *re-* means back or again, and since the foods never were in the body before, the term is hardly appropriate.

the pylorus into the intestine. Gases, like carbon dioxid, however, are absorbed. Alcohol, mono- and diglucids, like sucrose and maltose, are, in smaller quantities, dextrin, pepton, and neutral fats, are also absorbed by the stomach walls. However, this absorption is insignificant compared to that in the intestine.

Absorption in the Duodenum.—The duodenum[1] partakes of the dual function of digestion and absorption. It is the widest portion of the small intestine. The surface is highly specialized for absorption, the mucous membrane being thrown into folds known as valvulæ, which, in turn, have small finger-like villi on their surfaces. The folds are permanent and do not vary with expansion or contraction of the lumen of the duodenum. The villi, however, are reduced from 1 to 0.5 mm. as the duodenum becomes distended. The fact that the villi occur more abundantly in the duodenum than in the jejunum and ileum leads to the inference (for which there is abundant proof) that the function of absorption is primarily in the duodenum. The villus is supplied with two circulatory systems: (1) The blood system, whereby a small artery delivers blood to the base of the villus, whence smaller and smaller arterioles and capillaries distribute the arterial blood throughout the organ to its tip. After gaseous exchange in the villus the arterial blood is collected by a capillary network connected with the venous system. The vein leaves the villus at its base near the entrance of the artery. (2) The lymphatic system, represented by a lacteal, which usually consists of a single axial lymphatic vessel, the lacteal proper, communicating with a loose lymph-plexus lying just within the muscular coat of the villus. Special smooth muscle-fibres running lengthwise of the villus propel the fluids in the lacteal. The surface of the lacteal nearest the lumen of the intestine is composed of goblet and columnar cells through and between which the digested foods in the duodenum pass. This is setting for the transfer of digested foods from the alimentary canal to the lymph and blood system.

Absorption in the Jejunum and Ileum.—The villi are longest and most conspicuous in the duodenum and become progressively smaller and less significant in the jejunum and ileum, and finally disappear in the terminal ileum. The jejunum and ileum have many feet of absorptive surface, whereas the duodenum has only about 10 inches.

[1] Latin, *duodeni*, "twelve-at-one-time." Pronounced "duo-dē'num." Ignorant persons sometimes pronounce the word "duod'enum," an etymological impossibility. The twelve refers to the average length of the duodenum—equal to the width of that number of fingers laid side by side.

The proportion of food absorbed by each of the three portions of the small intestine is not known. The numerous villi[1] in the duodenum indicate rapid absorption, and the greater part of total absorption must occur there but the jejunum and ileum, since they have a much larger absorptive surface play a considerable rôle.

What Substances Are Absorbed?—We have said that a small amount of fat is absorbed by the stomach and the same is true of the intestine. Other colloids, like albumin, are also absorbed as shown by filling an isolated loop of the intestine with serum of the blood from the same animal; both crystalloids, such as inorganic salts, and colloids (like serum albumin) are completely absorbed into the blood, which must be left undisturbed in making the ligation isolating the loop of the intestine. However, this is of no special importance, the substances usually absorbed being crystalloids of lower molecular size. The monoglucids such as glucose are absorbed more rapidly than the diglucids like cane-sugar, and under normal circumstances diglucids are not absorbed until they are hydrolyzed to monoglucids—glucose, fructose, and galactose. When taken into the alimentary tract in large amounts the diglucids act as inorganic salts and attract water, thus exhibiting a laxative effect. Similarly, inorganic substances exhibit differences in absorbability, sodium chlorid passing from the intestine into the blood more quickly than sodium sulphate. Chiefly, this is a matter of the valence of the substance, the higher the valence, the lower the absorption, as exhibited by the monovalent ion Cl^- as contrasted with the bivalent $SO_4^=$ ion just mentioned. The low absorbability of the sulphion is utilized in laxatives, like magnesium with sulphion, Epsom salt, $MgSO_4$; while Epson salt has additional properties in purgation, the fact that the salt is absorbed but slowly insures that water will be extracted from the blood into the lumen of the intestine; this aids catharsis.

How are Substances Absorbed from the Intestine into the Blood? This question has never been satisfactorily answered. We may briefly summarize the salient points which have been determined: (1) Absorption is not simple diffusion; absorption must proceed sometimes from higher concentration to lower. Thus, the normal salinity of the blood is about that of a NaCl solution of 0.9 per cent. strength. If a loop of the intestine is filled with a NaCl solution in strengths not greater than 2 per cent., water will pass into the blood. However, from an NaCl solution greater than 2 per cent. water is not absorbed

[1] The movement of food through the duodenum is rapid normally, as determined by fluoroscopy.

into the blood, but passes into the intestine. Magnesium sulphate given along with drugs or after their oral administration causes retarded absorption of the drugs. (2) When substances are absorbed into the blood the tissues utilize more oxygen; that is, work is performed in absorption. (3) There is a selective action on the part of the membranes. Certain substances are absorbed much more quickly than others and in some cases absorption is almost suppressed. Thus NaCl passes from the intestine into the blood more quickly than compounds having the sulphion $SO_4^=$, as in $MgSO_4$, as we have mentioned, page 490. Water is absorbed into the intestine from the blood, thus facilitating the passage of the feces. Again, glucose is absorbed much more quickly than sucrose, and it is easily possible to take so large an amount of diglucids, such as sucrose, lactose, etc., that they act similarly to magnesium sulphate, causing the passage of water from the blood into the intestine. This selective action is lost when tissues are injured, as occurs by adding a small amount of sodium fluorid, which is a cell poison.

Fig. 158.—A. P. Mathews, Professor of Biochemistry, University of Cincinnati, Cincinnati, Ohio. Author of the well-known Physiological Chemistry. Contributor to the dynamics of biochemistry, absorption, adsorption, and to certain phases of descriptive biochemistry, such as lecithin, etc.

Certain characteristics of the process of absorption are noteworthy: (1) Absorption normally takes place from electrolytic solutions such as the digested products, protidtemns, lipidtemns[1] and glucidtemns, along with inorganic substances. The colloidal, nondiffusible substances present in the intestine are insignificant. (2) Absorption products pass into a typically colloidal solution, the blood, or, at first, into the lymph which, while lacking the erythrocytes, yet contain a high percentage of protid. (3) Absorption is ordinarily an irreversible process, that is, absorption goes to completion if the sub-

[1] The fatty acids are insoluble in water, being diffusible by aid of their solubility in the lipids composing the animal membranes.

stances are absorbed. (4) Complete absorption from the intestine is due to the blood constantly removing the absorbed materials. The case is similar to a chemical reaction in which the products are not permitted to retard its progress.

With these characteristics in mind, we may utilize a principle of modern physical chemistry which we have employed before[1] for similar purposes, namely, the Donnan equilibrium. This involves an unequal distribution of ions which are freely diffusible through a membrane when it has upon one side substances which do not diffuse, or do so reluctantly, like colloids. The blood contains non-diffusible serum globulin and albumin, and these prevent ions from diffusing into the intestine, while the ions are free to pass from the intestine into the blood. The movement of the blood carries these substances with it, making room for others.[2] Besides protids, other non-diffusible materials exist in the blood, and all act like colloids in preventing the passage of ions. The process is not, therefore, dependent upon the osmotic pressures, but upon selective diffusion, etc. Those substances which diffuse rapidly, such as NaCl, are quantitatively removed by the circulation, while those which diffuse more slowly, like $MgSO_4$, serve to hinder the diffusion of ions of similar sort.

It must be recalled that the digested food materials are electrolytes. Examples of these are amino-acids, fatty acids, and monoglucids like glucose, which are known to assume different forms under different conditions as revealed by the polariscope. The glucids are not well understood as far as diffusion is concerned. In each case we have substances which ionize into a large anion and a cation like H^+, Na^+, etc. Thus the amino-acid glycin ionizes as $CH_2NH_2.COO^-$ and H^+. In an alkaline medium all such substances bear negative charges which is proved by their wandering toward the positive pole in an electric stream, such as exists in the blood. Hence we can assume that there is a tendency toward the diffusion of anions into the blood. But what of the cations? As far as cation H^+ is concerned, it has been shown by Loeb[3] that hydrions preponderate outside a membrane where anions within the membrane are non-diffusible, as in the case of the protids of the blood. Hence we are able to erect a plan for absorption based upon modern theories of physical chemistry. The simplicity of this explanation has appealed to some as inadequate to

[1] Page 104.

[2] Macht, D. I. (Pharmacologist, Hynson, Wescott and Dunning, Baltimore, Md.), has shown that slowly moving currents favor absorption over rapidly moving fluids.

[3] Page 107.

show how substances pass through several different membranes like the intestinal endothelium, the muscular and parenchymal layers of the villi, and the endothelium of the capillary of the latter. It must be remembered that in each case similar factors are involved, and the passage from the alimentary canal to blood in a series of events each identical with the whole phenomenon as far as mechanism is concerned.

Where the Different Foods Are Absorbed.—*Glucids* are absorbed directly as monoglucids like glucose into the capillaries of the villi. While diglucids, like sucrose, are crystalloids, and therefore cass through membranes, yet the rate of absorption is low because of their molecular size, and they must be hydrolyzed to their component monoglucids to pass readily through the walls of the intestine into the blood. This is a point of great importance in tolerance tests,[1] for normally 320 gs. of sucrose must be taken by mouth before glycosuria occurs, whereas the ordinary test with glucose injected into a vein shows that 100 gs. may cause the appearance of glucose in the urine.[2] It is easy for a normal person to take enough lactose to cause diarrhea by the same mechanism as in the case of saline cathartics. Maltase does not occur in human blood, and for this reason injected maltose exists in the human blood for some time. Of the monoglucids, gluose is absorbed most quickly, then galactose, and finally fructose.[3] Just before death fructose is demonstrable in human blood; it disappears about the time of birth. Pentoses are absorbed by the alimentary tract. Negano[4] has studied the regions of the alimentary tract where absorption of glucids takes place.

Lipids differ from the glucids (and protids) in that their products are absorbed into the lymphatic system by the lacteals. The lymphatic system pours its fluids into the subclavian vein near the heart, thus obviating passage through the liver. Other substances, like glucidtemns and protidtemns, enter the portal system and go to the liver. Some fat passes into the portal system, but 60 per cent is taken up by the lacteals and carried to the blood by the thoracic duct. Unlike other substances, fat is resynthesized in the epithelium of the intestine from the products glycerol and fatty acids which are formed during digestion. Again, there is evidence that some unal-

[1] This means the amount of sugar which is just insufficient to cause glycuresis (see page 768).

[2] Page 768.

[3] Hewitt, J. A. (King's College, London). See Biochem. Jour., vol. 18, p. 161, 1924.

[4] Nagano, J. (Breslau), Pflüger's Arch. fuer die gesammte Physiol., vol. 90, p. 389, 1902.

tered fat is transported into the lymphatic system either by the activity of the epithelial cells of the villus, or by leucocytes which pass (diapedesis[1]) into the lacteal spaces in the villi. How much fat in the form of "fat dust" or minute globules of fat pass into the lacteal spaces is not known. The lacteal lies as an irregular space in the middle of the villus and fat globules temporarily accumulate in the epithelial cells of the intestine, in the subepithelial spaces, and in the cells surrounding the lacteal; but there is a constant flow of these fat globules into the collecting spaces which are in communication with the cisterna chyli and thence into the thoracic duct which flows into the subclavian vein.

Fats are transported in the blood as:

(1) Neutral fat.

(2) Hydrolyzed fat:

(*a*) Glycerol esters of two fatty acids, phosphoric acid and cholin (lecithins). Chiefly in the erythrocyte.

(*b*) Cholesterol esters of fatty acids.

Some soap is formed by the alkali of the intestinal juice and the fatty acids from digested fat. They aid in the emulsification of the fat by lowering the surface tension, permitting the particles to become finely divided. Unsaturated fatty acids are more rapidly absorbed than the saturated ones and the same is true of the fats themselves.

The passage of fat from the blood into the tissues involves un-hydrolyzed fat, there being no lipases in the tissues capable of hydrolyzing the higher fats and oils. Esterases, capable of hydrolyzing lower esters are characteristic of the tissues.

Protids.—Thanks to the discovery by Abel, using his vividiffusion apparatus,[2] and to Folin,[3] we know the manner in which protidtemns are absorbed. Typically these are amino-acids, but probably there is absorption of some of the lower peptids. Albumoses are toxic and, moreover, they are colloidal in nature and are not absorbed. Pepton possesses harmful effects[4] when introduced into the blood, for the coagulation time is prolonged, a fall of blood-pressure occurs, and

[1] Greek *dia*, through, and *pede*, a shackle; hence a boot, from which the meaning to progress.

[2] For illustration, see Macleod (page 130), 1918 ed., page 607.

[3] Page 19. See the series of papers Protein Metabolism from the Standpoint of Blood and Tissue Analysis, Jour. Biol. Chem., vol. 11, p. 87; vol. 12, pp. 141, 253, 1912. Non-protein Nitrogen of the Blood in Health and Disease, Physiol. Revs., vol. 2, p. 460, 1922.

[4] Pepton "shock" of the pharmacodynamic laboratory is due to albumoses found in the so-called Witte's "pepton" which is largely albumose. See page 496.

other pharmacological effects are manifest. Moreover, pepton injected into the blood is excreted nearly quantitatively. We should not expect pepton to be absorbed in any quantity. In fact, pepton is not found in the blood. Amino-acids pass readily through the membranes and are carried to the liver by the portal system. Unlike the lipids, no synthesis occurs until the amino-acids reach the tissues. Absorption takes place through the blood capillaries in the lacteals, similarly as in the case of the glucidtemns.

The manner of absorption of the products of digestion may be summarized as follows:

(1) Glucids and protids into the blood directly.

(2) Lipids into the lymphatic system and then into the blood.

The reason for this difference in manner of absorption probably depends upon the formation of fat emboli[1] in the blood-stream under certain conditions. These masses of fat find their way into the smaller vessels of the coronary circulation of the heart, the capillaries of the lungs, or into smaller vessels of the brain in which they may occlude the blood-vessels and cause death. To obviate any sudden addition of fat to the circulation, especially where the movement of the blood-stream is not rapid, the fat is sprayed into the rapidly moving stream near the heart.

DEMONSTRATIONS

The following Demonstrations concerning absorption are made in order that some of the chief factors in this process may be visualized:

1. Experimental Lipemia.—Feed a cat or a white rat for a day or more previous to the demonstration a high fat diet (steak with suet ground up with it and the whole fried). Place the cat under a bell-jar and by means of a rubber tube pass a current of illuminating gas into the jar. After death, stretch the animal upon a board and perform laparotomy. The lymphatic system is readily traced owing to the engorgement of the vessels with white chyle. Expose the thoracic duct and its termination in the precava. Remove some of the blood before it has clotted and add a small amount of potassium oxalate to prevent clotting. Place the blood in a 15-ml. centrifuge tube (Fig. 220) and centrifuge at moderate speed. Note the collection of creamy material on top of the various zones in the tube; this zone is fat. The Demonstration may also be made as follows: Feed the cat butter stained with a fat-staining dye like scarlet-red,[2] and repeat the operation as before. The presence of the stained oil through the

[1] Greek *embolos*, a plug.

[2] Page 199, note 3.

body is indicated by the pinkish tinge imparted to the tissues. The oxalate may be omitted in the centrifuge experiment, the blood being defibrinated by beating it with a small bundle of wires or small "pipe-cleaning brush" sold in the tobacco shops; the clot is removed and the serum centrifuged. The reddish or pinkish layer of stained fat is then readily detected. This condition is clinically known as lipemia. It appears in diabetes mellitus (Chapters XV and XVI).

2. Retention of Diglucids in the Intestine.—Select two white rats of similar weight and appearance. Paint the back of one with a dye or otherwise distinguish it from the second rat. Feed the marked rat a bolus of 2 gs. lactose in a piece of steak or gelatin capsule. Feed both rats plentifully of bran mixed with boiled white of egg. After fifteen minutes place the rats in different cages and note the character of the feces in each case. The marked rat should exhibit stools of loose consistency, due to the cathartic action of the non-absorbed lactose. Since both rats have been subjected to the same conditions other than the feeding of lactose, this sugar is the cause of the diarrhea.

The laxative effect of lactose may also be readily demonstrated upon the human subject.

3. Albumose Shock.—This Demonstration may be made by either the physiology or the biochemistry department. Appoint (*a*) surgeon, (*b*) assistant surgeon, (*c*) anesthetist, and (*d*) technician from the members of the class.[1] Select a rabbit and anesthetize it as follows: Lay the animal on its back in your lap and force between its jaws a thin flat stick with a hole in the middle large enough to admit a small "male" catheter to serve as stomach-tube. Measure the length of the tube from the rabbit's teeth to the middle of the stomach and mark the distance with ink upon the tube. Lubricate the tube with vaselene and pass it carefully up to the mark which will indicate that the tip of the tube is in the rabbit's stomach.[2] Now insert a small "physiological" funnel holding about 10 mls. of liquid into the free end of the stomach-tube and slowly pour into it a solu-

[1] In the case of large classes several groups may be selected each to perform this Demonstration.

[2] Inadvertedly the stomach-tube may enter one of the bronchi, in which case froth will appear at the open end of the tube and the froth will pulsate with the breaths. Lung edema will almost certainly follow and the rabbit should be abandoned. In order to insure against the tube entering the trachea proceed as follows: Lay the tube on the table and note which way it curves; than pass the tube so that the arc it forms is concave to the animal; this will insure that the tip is directed in its passage toward the dorsum and runs little chance of entering the trachea.

tion of urethan.[1] After half an hour apply to the rabbit's nose an anesthetizing ether cone. Let the animal inhale as long as there is an eye-reflex, which is determined by passing your finger over the cornea; blinking is continued until very deep narcosis occurs. Tie the animal upon an operating board. With scissors clip away the fur from the ventral surface of the neck and make a longitudinal incision along the median line. Expose the trachea by means of a blunt instrument like the handle of a scalpel; locate upon one side of the neck the carotid artery by means of its pulsations and clear the tissues from a part of it, avoiding injuring the nerve which passes in the same sheath. Pass silk ligatures beneath it, but without including the nerve, at either end of the cut. Now ligate distally, that is, farthest from the heart, and then place a hemostat as far proximally (toward the heart) as possible, in order to isolate a segment of the artery. By means of round-pointed scissors make a V-shaped cut across the artery in the swollen portion and hold the angular tab of the cut wall by means of forcepts while your assistant introduces a cannula and ties it in place by means of a second ligature, previously laid under the vessel. Fill the cannula with physiologically normal saline[2] and attach to the tube coming from a manometer which contains magnesium sulphate solution and which records its movements upon smoked paper fixed to a slowly rotating kymograph.[3] Now remove the hemostat from the carotid and the pressure of the blood will cause the manometer record to rise to a certain height. Permit the drum to revolve until a short tracing has been made; this is the level of normal blood-pressure. Inject, slowly, by means of a hypodermic syringe either into the femoral vein or into the jugular vein of the opposite side of the neck 2 mls. of 10 per cent. Witte's pepton[4] per kilogram of rabbit's weight. Note the fall of blood-pressure. Injection of more of the "pepton" causes a still further lowering of the blood-pressure.

Treatment.—The fact that the animal responds but slowly to agents used to raise the blood-pressure indicates the severity of the trouble and that it is most improbable that protidtemns are absorbed into the blood in any quantity in the form of substances higher than the amino-acids. (1) Inject into the vein 1 or 2 drops of 1:1000

[1] For dosage see Appendix. [2] Page 122.

[3] For illustration see Fig. 188, page 613, where the kymograph is used in connection with another form of apparatus.

[4] This form of reagent must be used. American pepton contains an extremely small amount of albumose.

epinephrin solution[1] which normally should cause a rise of blood-pressure; note the slight effect. (2) Repeat, using 5 drops of epinephrin.

4. Absorption of Inorganic Substances.— Using the same animal while continuing anesthesia, expose about a decimeter of small intestine and throw ligatures around each end, thus making an isolated loop, but leaving the blood-supply intact. Inject into the loop 10 mls. of 1 per cent. potassium ferricyanid solution. Then after five minutes draw 2 mls. of blood from the jugular or femoral vein and permit the blood to clot. Centrifuge at low speed and then decant the supernatant serum. Dilute the serum with 1 volume of water and add to it, drop by drop, ferric chlorid 0.1 per cent. solution. In the presence of ferric iron like ferric chlorid, Prussian blue[2] is formed, recognizable by its color.

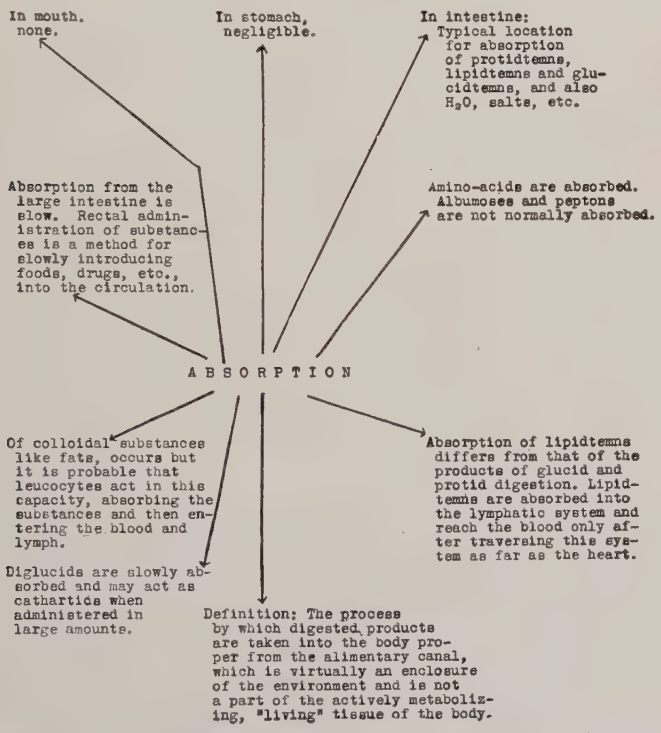
Absorption from the Large Intestine.—Man is intermediate between herbivorous and carnivorous animals in regard to the length of the large intestine and its accessories, cecum and appendix. Herbivores are characterized by the great length of the intestine, carnivores, by the shortness. The intestine of the sheep, as a typical herbivore, is twenty-seven times the length of the body, while in man it is only about one-fourth that, or seven times the body length. On the average omnivorous diet of man the large intestine has little to do, the cellulose content of the foods being relatively low as compared to that of the herbivore, and the digested foods are absorbed in the ileum or higher in the small intestine. If, however, the diet contains much cellulose (as one consisting of spinach, lettuce, cabbage, bran, etc.), the undigested residue enters the large intestine and will not behave as in a typical herbivore where the cellulose becomes hydrolyzed by cytase, a cellulose-splitting enzyme, and the products become absorbed into the blood from the walls of the large intestine. Such an enzyme does not occur in man. Bacteria, however, cause hydrolysis and other changes in the foods in the large intestine and the products are absorbed. Rectal feeding is resorted to in cases of stricture of the esophagus or pylorospasm, or in other conditions in which food cannot be taken through the usual channels; but absorption is a much slower process in the large intestine than in the small. Whereas almost unlimited quantities of glucose are absorbed by the small intestine, the large intestine absorbs only 6 gs. per hour. Water, likewise, is absorbed slowly, and this is a protective measure, for water must be left in the large intestine for the normal removal of

[1] Page 267.

[2] The reaction is: $K_4Fe(CN)_6 + 4FeCl_3 = Fe_4.[Fe(CN)_6]_3 + 4KCl + 4Cl_2$.

the feces. In fact, one of the great problems of mankind, especially in civilized life, is to furnish enough water and undigested food residues to the large intestine to cause it to function properly. The low power of absorption from the large intestine is utilized in therapeutics when it is desirable to administer a certain medicine which is absorbed slowly. On the other hand, absorption of decomposed products rom the large intestine leads to more or less severe illness, which may be relieved by enemas or by purgation.[1] Suppositories containing astringent medicines are frequently used in counteracting hemorrhoids and the absorption of such substances sometimes is undesirable. The excretory function of the large intestine is of great importance. Substances like bismuth taken to counteract diarrhea are excreted through the walls. In case of mercury poisoning, colonic irrigation serves to remove some of the mercury that has been excreted into the large intestine.

GRAPHIC SUMMARY


In mouth. none.
In stomach, negligible.
In intestine: Typical location for absorption of protidtemns, lipidtemns and glucidtemns, and also H_2O, salts, etc.
Absorption from the large intestine is slow. Rectal administration of substances is a method for slowly introducing foods, drugs, etc., into the circulation.
Amino-acids are absorbed. Albumoses and peptons are not normally absorbed.
ABSORPTION
Of colloidal substances like fats, occurs but it is probable that leucocytes act in this capacity, absorbing the substances and then entering the blood and lymph.
Absorption of lipidtemns differs from that of the products of glucid and protid digestion. Lipidtemns are absorbed into the lymphatic system and reach the blood only after traversing this system as far as the heart.
Diglucids are slowly absorbed and may act as cathartics when administered in large amounts.
Definition: The process by which digested products are taken into the body proper from the alimentary canal, which is virtually an enclosure of the environment and is not a part of the actively metabolizing, "living" tissue of the body.

[1] However, not all of the alleged ills attributable to absorbed intestinal products are justifiably assigned to these substances. Impaction of sterile gauze into the rectum will cause similar effects, such as headaches. See Alvarez (page 500). Also Donaldson, A. N., Jour. Amer. Med. Assoc., vol. 78, page 884, 1922.

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CHAPTER XI

THE FATE OF THE ABSORBED MATERIALS; INTERMEDIATE METABOLISM

"After long and tiresome wandering, this pass is attained; from which we may hope to advance some little way perchance into the broad mysterious domain of metabolism."—*Von Fuerth.*[1]

The digested and absorbed food substances, the glucidtemns, lipidtemns, and protidtemns, have a common fate—oxidation and excretion. Some of the substances may become incorporated into the structure of the body and their oxidation and excretion be delayed temporarily, but sooner or later they suffer the common fate of all: Excretion as CO_2, H_2O, ammonia, or other products. The term "assimilation" signifies to make similar, but we know little concerning the manner in which the amino-acids, glucose, glycerol, and the fatty acids enter into the living protoplasm, or, indeed whether there is any configuration which may be termed "alive" other than the sum total of the various substances.[2] Speculation concerning "living molecules" may serve to while away an idle hour, but can scarcely clarify the understanding of the workings of the organism, normal functioning of which it is the duty of the physician to maintain in health. Let us turn to a more practical consideration —the manner in which the various substances absorbed from the alimentary tract into the blood are utilized. It must be borne in mind that there is still much to be explained regarding their ultimate disposition.

Oxidation in the Organism.—The dependence of vital activities on air (oxygen) is common knowledge, but the manner in which oxygen functions in respiration is unknown. Within the body, a substance is readily oxidized which, in the test-tube requires great heat. Thus, one gram molecule of glucose is burned in the test-tube when 677 calories of heat energy are supplied, but the sugar is readily oxidized in the body at 37° C. The question is, what addi-

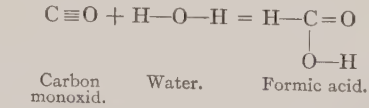
[1] Von Fuerth, Otto, Austrian Biochemist, Vienna, Chemistry of Metabolism. (Translated by Smith, University of Pennsylvania), Philadelphia, Lippincott, 1916.

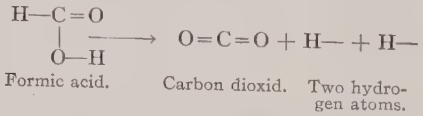
[2] For a discussion see Verworn, M., Die Biogenhypothese, Jena, Gustav Fischer, 1903. Also Moore, B., Biochemistry, London, 1919. See also page 220.

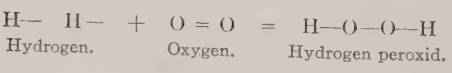
tional factor is present in the organism to permit glucose to burn at body temperature.

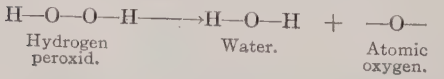
Briefly stated, the modern view of oxidation in the body is as follows: The *function* of oxidation is primarily to remove products of metabolism and secondly to furnish that amount of heat necessary to maintain the body temperature near the optimum for enzyme action. We know that the body may continue activities for some time without oxygen being supplied and that in the meantime the body is not depending upon the assumed "intramolecular oxygen" once believed to account for such observations, but rather that the body overdraws its account for oxygen and the deficit is made up when oxygen is supplied. Specifically, muscle-action is due to the conversion of glycogen into lactic acid (page 145) and after the muscle contracts, the excess acid is removed by oxidation, which at the same time provides the heat energy necessary to maintain the normal temperature. Now the formation of lactic acid may proceed for some time and not be removed by oxidation, as in strenuous exercise; but later oxygen must be supplied and then there is a large excretion of products of combustion due to the burning of the accumulated acid.

As to how oxygen behaves in combustion, only theories are available at present. Water is known to be necessary. Carbon is burned in the test-tube according to the following reactions: (Using carbon monoxid)

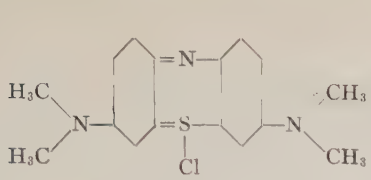




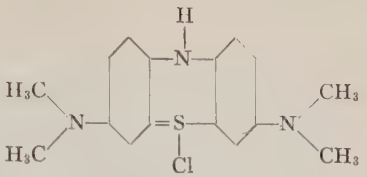




The essential steps are (1) hydration, (2) production of hydrogen (3) transport of hydrogen to another substance, (4) formation of a peroxid and, lastly, (5) the production of atomic oxygen. In the above reaction, oxygen acts as a receiver of hydrogen, but other substances may serve in this capacity, as, for example, methylen blue



Methylene blue.



Leuco-methylen blue.

which becomes reduced to the leuco-compound; metadinitrobenzene, which becomes reduced to a yellowish compound metanitrophenylhydroxylamin and lastly, substances which occur in the normal body, among which the dipeptid glutathion (page 343) is at present extensively studied. Mathews (page 491) a number of years ago showed that some sulphydryl compounds were capable of "spontaneous" oxidation (autoxidation), this property being destroyed by cyanid poisoning. Minute traces of iron accelerate such oxidation. Glutathion is doubtless the substance responsible for the furnishing of hydrogen to methylen blue when this substance is brought into contact with live tissues; the methylen blue is reduced to the leuco-substance. Whether thyroxin may act as hydrogen acceptor or not remains unanswered. In the case of glutathion, the acceptor is maintained in the body in a reduced condition owing to the reducing action of the tissues; as fast as it is oxidized by oxygen of respiration, forming the tetrapeptid, it becomes reduced to the dipeptid condition. The substance in the tissues responsible for reducing glutathion is unknown, but Hopkins and coworkers have shown that the tissue may be washed, boiled, extracted with ethanol and dried and still retain the property of causing reduction. One gram of this tissue residue reduces 6.5 mgs. of oxidized glutathion.

In summary, we may give the following scheme for tissue oxidation, using glutathion as hydrogen acceptor and a fatty acid as capable of forming the peroxid:

$$RH_2 + GS{-}SG = R + 2GSH$$

Tissue residue 1 g. — Glutathion oxidized 6.5 mgs. — Tissue residue (oxid). — Glutathion reduced.

Active hydrogen is then produced, probably by respiratory oxygen acting as acceptor of the hydrogen:

$$2GSH \xrightarrow{+O_2} GS{-}SG + H_2O_2$$

Glutathion reduced. — Glutathion oxidized. — Hydrogen peroxid.

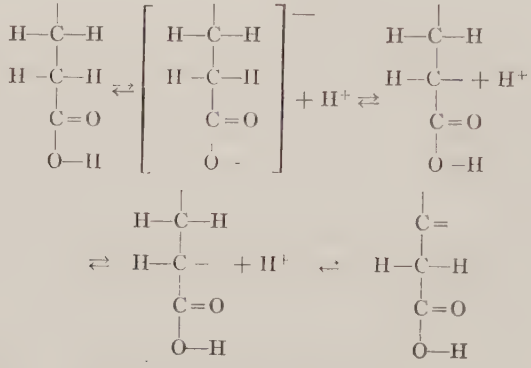
Any substance capable of accepting oxygen will then become oxidized:

$$H{-}O{-}O{-}H \longrightarrow H_2O + \tfrac{1}{2}O_2$$

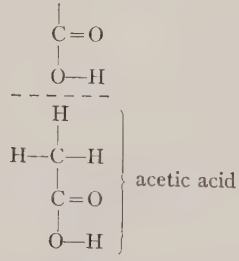
Peroxid. — Nascent oxygen.

Lecithin and unsaturated fatty acids like linolenic (page 202) readily take up oxygen.

In the foregoing discussion, we have considered indirect methods of oxidation. Quastel[1] suggests that polar groups (page 115) may be activated at cell phase boundaries (page 99) and the substance may become activated; thus—



β-oxidation, page 526, will then occur in the presence of water:



Lastly, enzymes have been called upon to account for the rendering of molecular oxygen active. A catalase capable of producing atomic from molecular oxygen is present in many tissues. Such a catalase occurs in the potato and in blood (page 118). If a substance acting as an indicator of active oxygen by change of color, like guaiacum, be brought into contact with potato parings, the guaiacum becomes blue. The rôle of peroxidase is to split, from an autoxidizable substance, forming a peroxid, atomic oxygen. Lecithin has been identified as a possible autoxidizable substance.

It is doubtful that the catalase is responsible for the oxidations of importance in metabolism.[2]

[1] Quastel, J. H. (Cambridge, England), Biochem. Journ., vol. 20, page 166, 1926.

[2] Quastel has calculated that if the specific enzyme hypothesis is valid, 56 specific oxidizing enzymes must occur in a bacterium!

THE UTILIZATION OF GLUCIDS

Glycogen Synthesis.—The liver contains about 300 gs. or 15 per cent.[1] of its weight and the muscles about 2 per cent. of the polyglucid, glycogen. All organs except the brain contain a certain amount. When glucidtemns, like glucose, fructose and galactose, and certain substances derived from them—glycerol, etc.—are absorbed, they are carried by the portal system to the liver, where some are converted into glycogen. When glycogen is called upon to furnish glucids for use in the body by reversed chemical process, only glucose is furnished. There is some evidence that glycogen may be utilized directly. It would give more heat per gram (4.2 Cals.) than glucose (3.74 Cals.). In fasting as much as 232 gs. of glycogen can be used, but it is not known whether glycogen is used as such in any case, or whether glucose is the intermediate product. Normally the glucose circulating in the blood amounts to about 5 gs. for the whole amount of blood in the body. The lactose of milk in the mammary gland is derived from glucose, but a high diet of galactose is more fully utilized by the female than by the male.[2] Berglund has shown that while galactose shows great individual variations in its behavior in different people, the tolerance[3] for a given individual is nearly constant. Above the limit of tolerance increase in amount of galactose fed is followed by increased excretion through the kidneys. None of these studies answers the question

Fig. 159.—Carl P. Sherwin, Professor of Chemistry, Fordham University, New York, N. Y. Investigator of the intermediate metabolism of substances, their behavior in the body, and their fate.

[1] This is about the percentage of starch in the legumes, like peas.

[2] Rowe, A. W., (Biochemist: Evans Memorial Hospital, Boston, and Professor, Boston University,) Jour. Biol. Chem., vol. 55, p. vi (Proceedings), 1923. Also Berglund, H., and Ni, Tsang G. (Harvard Medical School), Jour. Biol. Chem., vol. 63, p. xxxviii (Proc.), 1925.

[3] By this term is meant the amount beyond which the substance is not utilized. The tolerance for galactose (Rowe) for males is 30 gs. and for females 40 gs. by mouth.

whether galactose is utilized as such, or only through glycogen and glucose as intermediaries.

The D/N Ratio.— Besides the substances already mentioned as giving rise to glycogen, protids are capable of producing it. If an experimental animal be made diabetic[1] and placed on a purely protid diet after a period of fasting, glucose will appear in the urine because none can be used. By estimating the nitrogen excreted in the urine at the same time, the amount of protid metabolized during this period of experimentation can be estimated by multiplying the urinary nitrogen by 6.25 (see page 290) In this way it has been found that for every 100 gs. protid fed, about 58 gs. are convertible into glycogen. *Theoretically*, we may derive this figure as follows: The amino-acids are deaminized and the resulting fatty acids are converted into glycogen. Under the discussion of amino-acids[2] we have indicated which ones can enter into the formation of glycogen. We may summarize these statements by Lusk's[3] figures:

	Gs. amino-acid.	Gs. glucose.
Glycin	4.0	3.2
Alanin	8.1	8.2
Aspartic acid	10.6	7.2
Glutamic acid	22.3	13.6
Prolin	8.0	6.3
Arginin	11.5	5.9
Cystin		(undetermined)
Serin		
Total	64.5	44.4

About 60 gs. of amino-acids are convertible into approximately 45 gs. of glucose. The total nitrogen in 100 gs. of protid (beefsteak) is on the average 16.18 gs.[4] The ratio, therefore, of the amount of glucose to nitrogen in the urine:

$$\frac{\text{Grams of glucose from 100 gs. protid}}{\text{Grams of nitrogen from 100 gs. protid}} = \frac{44.4}{16.18} = 2.74.$$

This ratio, known as the D/N ratio (D standing for dextrose, equivalent to glucose) is very important. It represents the theoretical ratio, but actually, when no glucose is utilized in the organism, as in diabetes mellitus, 59 gs. of glucose are obtained from feeding 100 gs.

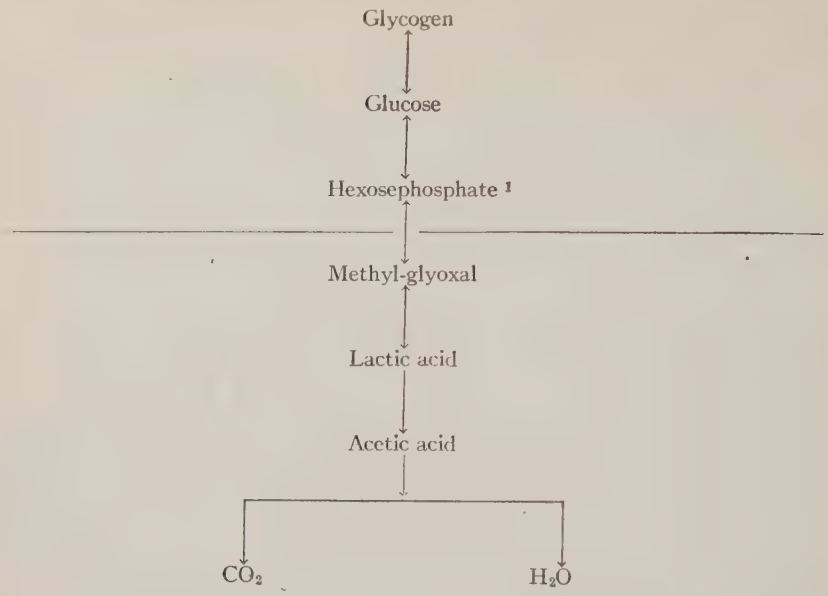
[1] The most convenient method is to administer phlorhidzin (page 922).
[2] Page 506.
[3] Lusk, Graham, page 24.
[4] Per cent. nitrogen in blood fibrin, 17.0; egg-albumin, 15.5; serum globulin, 16.0.

of protid, giving a ratio of 3.65. The additional amount of glucose is derived from some unknown source. The ratio 3.65 is a fatal ratio, since at this point the body has lost all its power to utilize glucids (and fats as well), and protid is furnishing the glucose which the diseased body calls from any source possible, only to waste it into the urine. In other words, the body substance itself is being wasted, similar to the loss in a furnace not only of all the fuel, but of the structure of the furnace itself. *The reader must understand that there is no means of determining a D/N ratio except in pathological cases because normally no glucose appears in the urine.*

Lipids to the extent of 10 per cent. of their weight, which represents their glycerol,[1] may be capable of forming glycogen in man. The point is still in doubt. Of the glucids, other than those mentioned, which form portions of the food of man, there are substances with fewer than six carbons, such as the pentoses. These are not digested by the secretions of the alimentary tract, but are released from their compounds, the pentosans or polypentids (the form in which the pentoses largely occur in plants), by the action of bacteria in the large intestine and are absorbed as pentoses and products capable of being synthesized, as least in part, into glycogen. The "normal glucid" content of the urine is chiefly pentose, so that it is improbable that much pentose is utilized by the human body. It is certain that pentoses are not utilized as such in the body, for when xylose or arabinose is injected into the blood-stream it is excreted quantitatively in the urine. It is only the products of pentose fermentation by bacteria that form glycogen.

The statement was made on page 508 that, roughly, those products of yeast fermentation of glucose which stand in the direct line from glucose to CO_2 and H_2O are also found in the normal metabolism of glucose in the body.

[1] Taking stearid as an example. Mol. wt. 891.1. The glycerol contained in the molecule is 92. The percentage of glycerol is, therefore, 891.1:92 = 100 : x = 10.3, or nearly 10 per cent.



The chief difference between yeast fermentation and the normal utilization of glucid in the body concerns lactic acid, which, according to Buchner and others,[2] does not occur in alcoholic fermentative processes. Methyl-glyoxal may be the starting-point for the synthesis of fat from glucids.[3] Nearly all tissues are glycogenic, but the nervous system is an exception. A glycogenolytic (glycogen-destroying) enzyme, however, is present. Of the two chief glycogenic tissues, liver and muscle, liver can utilize the substances below the horizontal line in the table above, but muscle cannot. Muscle requires glucose for the formation of glycogen.

Glycogenesis Depends upon the Normal Function of Other Organs, Especially the Pancreas, Thyroid, and the Adrenals.—Hypoglycemia follows extirpation of the liver after all muscle glycogen has been used up (Mann, page 474). The importance of the liver in glucid metabolism is proven by this observation. Extirpation of the pancreas likewise leads to profound changes in the metabolism of the sugars. The hormon of the pancreas is insulin. *Insulin*[4] probably has two factors: (1) One influencing the utilization of glucose in the body and (2) one depressing the process of glycogenolysis. When insulin is administered by mouth the former is inhibited, but the latter is left intact. The action (1) is probably that of increasing the permeability of the tissues for glycogen; glucose leaves the blood,

[1] See page 176 concerning the utilizability of the simplest ketose, dihydroxyaceton.
[2] Page 176. [3] Page 174. [4] Page 510.

or else becomes masked in some manner so that it does not give glucose reactions. It becomes inactivated when taken by mouth, probably through the reaction of the intestinal fluids; one Toronto unit[1] of insulin is inactivated by 0.003 mg. of trypsin. The insulin may be reactivated by adding acid to change the reaction from that of the intestine[2] to an acidity of at least *p*H 4.6. The factors inhibiting glycogenolysis (2) are unknown. What becomes of the glucose after leaving the blood is not known.[3] As stated above, it may not leave the blood, but becomes masked in some manner so that its reducing properties, which generally characterize it, are lost. It may form a union with phosphoric acid, producing hexosephosphate. Insulin injected into a normal animal produces glycogenolysis and disappearance of glucose from the blood. Injected into a depancreatized animal it produces glycogenesis when glucose is fed at the same time that the insulin is administered by hypodermic injection. The internal secretion of the parathyroid causes a reduction in glycogenolysis in an alkaline medium and thus produces deposition of glycogen. Insulin acts similarly when glycogenase of the liver is extracted and brought into contact with the glycogen of the blood, but the reaction must be acid or neutral.

Thyroid substance, under similar conditions, acts as a stimulant for glycogenase and glycogen is hydrolyzed to glucose; this occurs at the normal hydrion concentration of the blood, *p*H 7.3. There seems to be a synergy[4] between the parathyroid and pancreas respecting glycogenolysis. Insulin alone has no effect upon glycogen metabolism, but insulin plus parathyroid induces an "insulin" action, that is, causes the lowering of blood sugar in the normal animal and the deposition of glycogen in a depancreatized animal. It is possible that the chief function of insulin is to inhibit the action of the glycogen-cleaving enzyme, glycogenase of the liver, and muscles.

Pituitary substance[5] seems to antagonize this action of insulin and to aid glycogenolysis even in the presence of parathyroid sub-

[1] Page 513.

[2] Hume, H. V., and Denis, W. (Tulane University, New Orleans, La.), report the maximum reaction of the human duodenum as *p*H 8.23, minimum *p*H 5.9; average *p*H 7.02. See Jour. Biol. Chem., vol. 60, p. 633, 1924.

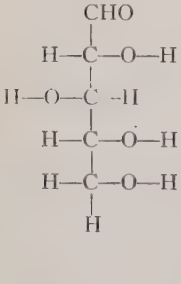
[3] Corís (page 376) have recently studied the fate of glucose under the influence of insulin by quantitative analyses of an insulinized and a control series of animals. They conclude that glucose is oxidized under the effect of insulin and that no unknown and hypothetical substance into which glucose is supposed to be converted need be postulated.

[4] Greek *syn*, together, and *ergon*, work.

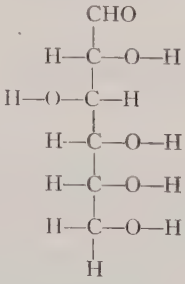
[5] Posterior lobe substance; see page 663.

stance. Cammidge[1] believes that the function of insulin in the normal body is to depress the glycogenase of the liver and other tissues, as we have already stated. True diabetes mellitus, then, may be defined as glycogenolysis unrestrained by insulin, which is not present in adequate quantities owing to damage to the pancreas. Such disease is typical of that type of diabetes mellitus which appears fairly suddenly in the young, as contrasted with a slowly developing type characteristic of older subjects, which is probably due to sclerotic conditions, or perhaps to an irregular interplay of endocrines, like the parathyroid (inhibiting hydrolysis of glycogen) and the adrenals (accelerating glycogenolysis). Of the two theories concerning the etiology of diabetes mellitus, (1) that it is due to lack of power of oxidizing substances like glucose, fats, etc., and (2) that it is due to overproduction of glucose by excessive hydrolysis of glycogen, unrestrained by insulin, the latter seems probable. One reason for believing that (2) is the actual factor, or at least a potent one, is that when insulin is injected the liver gives off less glucose, and at the same time, there is no loss of glycogen. This phenomenon occurs, however, only in the case of a damaged pancreas. Concerning (1) it may be said that insulin does not prevent the acidosis which occurs in diabetes by either mobilizing glucose or causing greater utilization of it. One gram of glucose per kilo of body weight admnistered to an animal increases the consumption of oxygen 20 per cent. Ten units of insulin[2] decrease oxygen consumption 15 per cent.

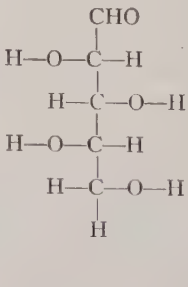
Diabetes mellitus is not concerned alone with glucose. Arabinosuria, galactosuria, and maltosuria respond to insulin administration. One should expect this to be true for the pentoses because of similar structure, which is evident from the following formulæ:



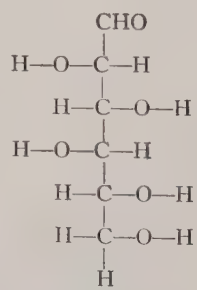
Pentose (arabinose).



Hexose (*d*-glucose).



Pentose (*d*-xylose).



Hexose (*l*-idose).

[1] Cammidge, P. J. (Physician, 32, Nottingham Place, London, W. I., England), widely known for his functional test for pancreatic efficiency, the "Cammidge Reaction."

[2] Toronto units, not per kilo, but for the whole body.

Hexoses are interchangeable in rather low alkalinity. Glucose and fructose are converted to a common unsaturated form known as the enol;[1] the complete reaction of such interconversion is given on page 172.

The Chemical Nature of Insulin.—It is probable that insulin is a polypeptid, a group of substances already definitely known to participate in important ways in the metabolism of the body.[2] However, unlike some of the polypeptids known to possess pharmacological activity, insulin gives the biuret test, indicating that at least three amino-acids must be present in the molecule.[3] The glyoxalic and Millon tests[4] are positive, showing that tryptophan and tyrosin are present. When analyzed by the method of Folin and Looney (page 254), there is found 17.9 per cent. tyrosin, 7.1 per cent. cystin, 0.8 per cent. tryptophan and 8.5 per cent. histidin.[5] Insulin is not digested by pepsin nor by trypsin, but in an alkaline medium in which trypsin is present, insulin becomes inactivated. It may be reactivated, however, showing that it is not destroyed.

Abel has crystallized insulin (see figure) and will be able to state the chemical composition of the pure compound.

Bertrand (page 140) has found that insulin contains a considerable quantity of nickel and cobalt, compared to the content of other organs.[6] The amount of sugar metabolized under the action of insulin is increased when nickel or cobalt is given simultaneously. Bertrand thinks that these substances may act upon a pro-insulin converting it to insulin; compare page 464.

Insulin is not responsible for all of the pancreatic hormonic function. If the pancreas is removed from a dog, it may be kept alive for some weeks by insulin injections, accompanied by feeding glucids yielding glucose, but sooner or later, according to the condition of the animal, it begins to lose weight and hepatic difficulties arise. If such an experimental animal be fed raw pancreas,[7] very beneficial results

[1] Pages 159 and 172.

[2] Compare glutathion, page 343. Abel has shown recently that insulin contains sulphur similarly as glutathion does.

[3] Page 285.

[4] Pages 312 and 241 respectively.

[5] Blatherwick, N. R. (Potter Metabolic Clinic, Santa Barbara, California), et al., Journ. Biol. Chem., vol. 72, page 57, 1927.

[6] The amount is never over a fraction of a milligram per kilo of tissue.

[7] See Chaikoff, I. L., Macleod, J. J. R., and Markowitz, J. (Toronto), Jour. Biol. Chem., vol. 63, p. lx (Proceedings), 1925. These workers find that if 50 gs. of raw pancreas, 200 to 400 gs. meat, and 50 gs. of sucrose be fed and at the same time two daily injections of 16 clinical units of insulin be given, an 8- to 10-kilo dog remains in good condition although the pancreas has been wholly removed.

are obtained. It is not known what substances are responsible for this amelioration. If the treatment is discontinued, three conspicuous changes occur: Increase in blood-sugar, appearance of aceton and hydroxy and keto-acids in the urine, and third, increase of phosphoric acid in the blood.

Glycogenolysis.—We have spoken of the belief of some biochemists[1] that glycogenolysis tends to occur at all times and that some agent, or agents, or combination of factors inhibits this action. The excessive hunger of fatigue and malaise experienced by some persons

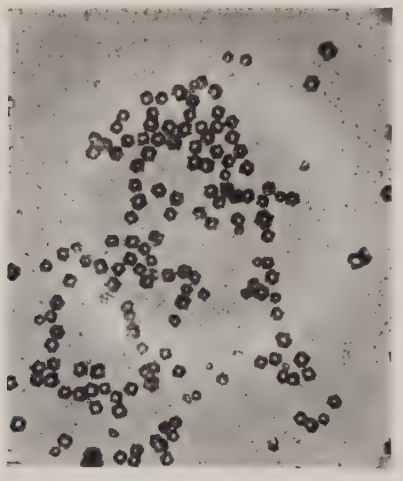


Fig. 159*a*.—Insulin crystals prepared by Dr. John J. Abel. Crystals precipitated from acetic acid solution with N/6 pyridine. (Courtesy of Dr. John J. Abel.)

during short periods of fasting may be due to excessive glycogenolysis caused by "hypo-insulinism," or underproduction of insulin in the body due perhaps, as Sevringhaus[2] suggests, to a fasting ketosis, which inhibits the production or action of insulin. The lack of glycogen in the nervous system, previously mentioned, is explained by the fact that an active glycogenase is present, which causes the hydrolysis of any glycogen entering that system. Certain factors related to the nervous system cause glycogenolysis. Epinephrin, which is influenced by the autonomic system, causes hydrolysis of glycogen. The piqûre[3] is another reaction related to the nervous sys-

[1] Page 510.

[2] Jour. Biol. Chem., vol. 63, p. xlviii (Proc.), 1925.

[3] Chapter XVII.

tem. A relation between blood phosphates and glycogenolysis has been established. When the piqûre is done, or epinephrin is injected, there is a sudden glycogenolysis and hyperglycemia, together with a fall in blood phosphates. Pituitrin, on the other hand, causes an antagonistic action to these two agents, and glycogen synthesis occurs along with a conspicuous increase in blood phosphates.

DEMONSTRATION.—*Glycogenolysis with Insulin.*—Inject into a normal rabbit 5 clinical units of insulin. Withdraw a sample

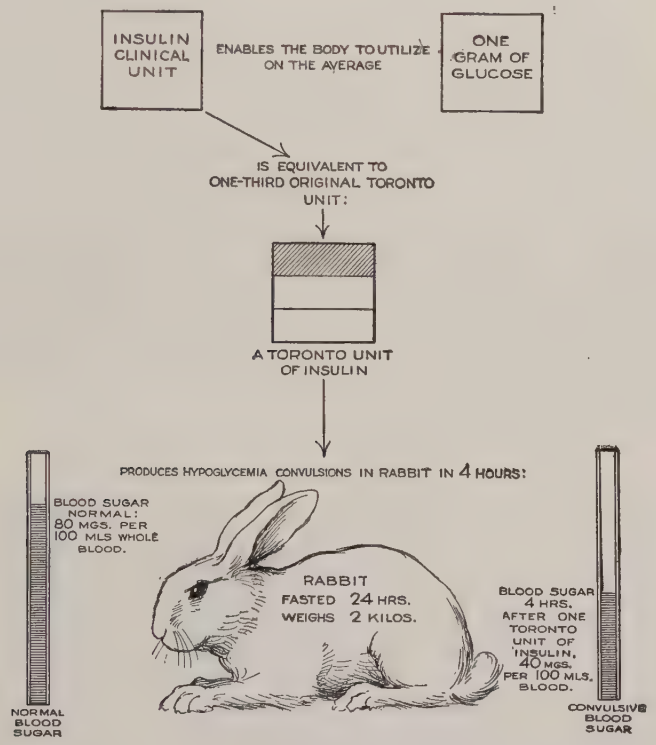

INSULIN CLINICAL UNIT
ENABLES THE BODY TO UTILIZE ON THE AVERAGE
ONE GRAM OF GLUCOSE
IS EQUIVALENT TO ONE-THIRD ORIGINAL TORONTO UNIT:
A TORONTO UNIT OF INSULIN
PRODUCES HYPOGLYCEMIA CONVULSIONS IN RABBIT IN 4 HOURS:
BLOOD SUGAR NORMAL: 80 MGS. PER 100 MLS WHOLE BLOOD.
RABBIT FASTED 24 HRS. WEIGHS 2 KILOS.
BLOOD SUGAR 4 HRS. AFTER ONE TORONTO UNIT OF INSULIN. 40 MGS. PER 100 MLS. BLOOD.
NORMAL BLOOD SUGAR
CONVULSIVE BLOOD SUGAR

Fig. 160.—Scheme to show quantitative relations of the insulin unit.

of blood from the marginal vein by snipping it with a pair of scissors. The instructor will determine the blood-sugar content.[2] Note the appearance of convulsions. Inject the femoral vein by means of a hypodermic needle and syringe with a small amount of glucose.

[1] The amount of glucose varies with the subject and with the condition of the subject. In mild human diabetes, 3–5 gs. of glucose are utilized per unit of insulin. In moderately severe cases, only about 2.5 gs. are utilized.

[2] By the method given in Chapter XVI.

Recovery from convulsions may or may not occur, according to the state of the rabbit.[1]

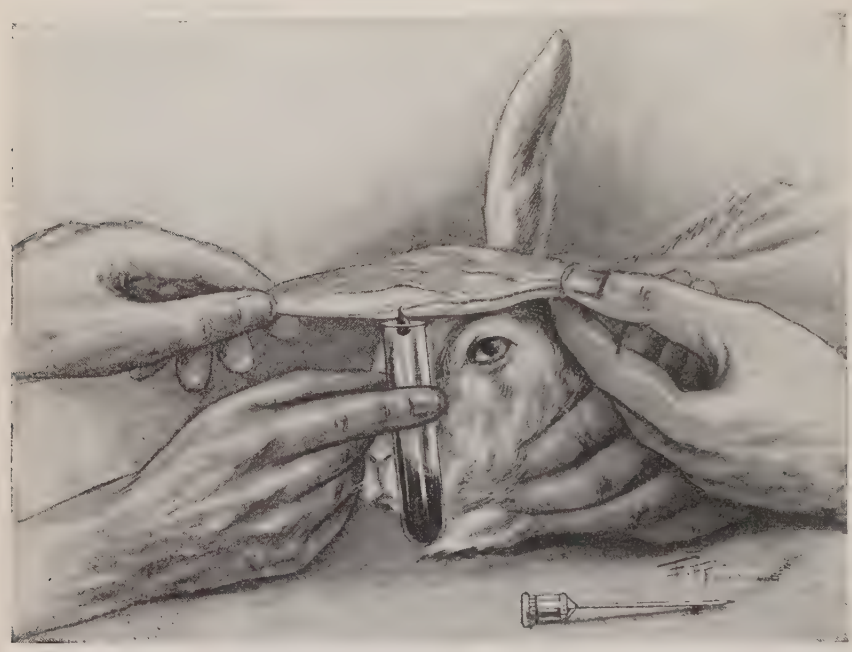


Fig. 161.—Method of bleeding a rabbit from the ear. A slit is made in the marginal vein. (From Kolmer, Infection, Immunity, and Biologic Therapy.)

Fig. 162.—Insulin shock in rabbit. The rabbit had been given the equivalent of two Toronto insulin units and went into hypoglycemic shock at the end of 2.5 hours. Recovery was made by administering glucose solution by stomach-tube.

[1] In order to avoid convulsions from too great lowering of the blood-sugar in clinical cases a small amount of glucose (see Fig. 160) is administered by mouth or by vein to a human subject.

DEMONSTRATION. *—Glycogenolysis Following Pancreatectomy.—* Anesthetize a rabbit as directed on page 190 and lay on an operating table as in Exercise 26. Perform laparotomy and locate the duodenum and pancreas, a yellowish diffuse gland lying against the former and lower portion of the stomach. Completely remove the pancreas, being certain that none is left. Ligate any bleeding vessels. Lay large hemostats parallel with the incision, clamp the lips together, maintaining anesthesia throughout. Catheterize the bladder and determine by Benedict's qualitative method[1] the presence of glucose in the urine. After glucose begins to appear, inject 5 units of insulin into the femoral vein and continue the sampling of the urine; the glucose will be eliminated after a time. However, if urinalysis is made at intervals for several days it will be found, as Macleod has demonstrated, that insulin does not prevent glycosuria indefinitely when all of the pancreas has been removed[2].

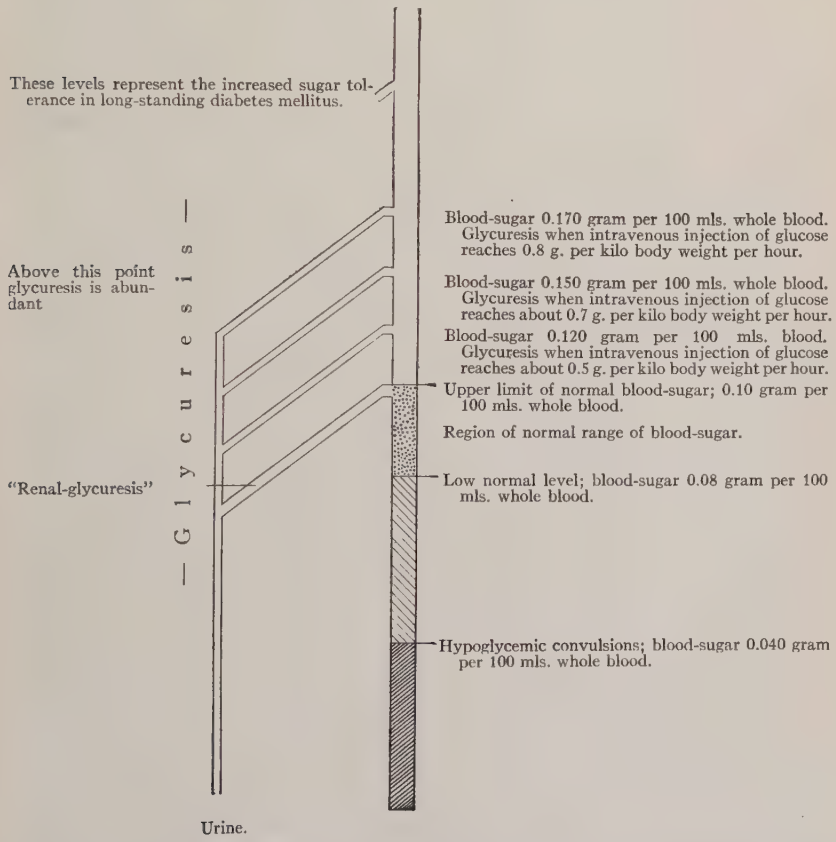

These levels represent the increased sugar tolerance in long-standing diabetes mellitus.
Above this point glycuresis is abundant
— G l y c u r e s i s —
"Renal-glycuresis"
Blood-sugar 0.170 gram per 100 mls. whole blood. Glycuresis when intravenous injection of glucose reaches 0.8 g. per kilo body weight per hour.
Blood-sugar 0.150 gram per 100 mls. whole blood. Glycuresis when intravenous injection of glucose reaches about 0.7 g. per kilo body weight per hour.
Blood-sugar 0.120 gram per 100 mls. blood. Glycuresis when intravenous injection of glucose reaches about 0.5 g. per kilo body weight per hour.
Upper limit of normal blood-sugar; 0.10 gram per 100 mls. whole blood.
Region of normal range of blood-sugar.
Low normal level; blood-sugar 0.08 gram per 100 mls. whole blood.
Hypoglycemic convulsions; blood-sugar 0.040 gram per 100 mls. whole blood.
Urine.

Chart showing the variations in blood-sugar while fasting. The chart is for glucose, but applies likewise to levulose. For galactose, or lactose, galactosuria or lactosuria varies as the square of the intake by mouth.

[1] Page 160. [2] Page 511.

Man may be made glycogen free by causing him to shiver; indeed, Carlson has shown that glycogenolysis following injection of insulin is total only when the stage of convulsive contraction of muscle, resembling shivering, is reached. Liver glycogen is removed without shivering, but muscle action is necessary to cause the removal of muscle glycogen. Experimentally in animals glycogen may be entirely removed from both liver and muscle by injecting 50 mgs. per kilo body weight of hydrazine sulphate, $H_2N—NH_2.H_2SO_4$. The cause of the reduction is increased utilization of glucids by the body.

Glycogenase, the Liver Amylase Hydrolyzing Glycogen.—This enzyme causes the conversion of glycogen into glucose immediately after the cessation of blood flow through the organs. This has been shown for the liver (page 190, Ex. 26).

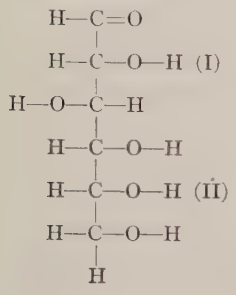
Insulin-like Agents.—On a previous page (page 260) we have referred to the insulin-like action exhibited by guanidin substances and especially one of the general nature of an amine of the amino-acid arginin which contains the guanidin radicle.[1] The substance referred to, "synthalin," is being synthesized artificially and has the advantage over insulin as an agent capable of reducing circulating glucose inasmuch as the synthetic compound may be administered by mouth. The new agent has decided limitations, however, and cannot replace insulin, especially because of the slowness of its action. It is about 100 times less potent than Abel's crystallized insulin (page 512). The maximum dose is that amount which enables the patient to metabolize 40–50 gs. of glucose. Its action is more prolonged than insulin and for this reason, it is unnecessary to administer the new substance every day.

Glycogenase is especially active in the heart muscle, all of the muscle glycogen being hydrolyzed into maltose and glucose within twenty-four hours after death, as contrasted with the much slower action of the enzyme in skeletal muscle, in which only about 10 per cent. of glycogen is hydrolyzed in that time. After death the disappearance of glycogen from these tissues runs parallel with the loss experimentally. In fact, if antiseptics like chloroform be used on recently dead muscle tissue, there is little or no hydrolysis of glycogen; such reagents injure the enzyme. Glycogenase follows other enzymes in being developed as growth occurs. In the embryo the rate of glycogenolysis is much slower than in later life. It seems, however, that

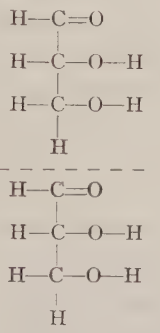
[1] See Frank, E., Nothmann, M., and Wagner, A. Ueber synthetisch darstellung Körper mit insulin artiger wirkung auf den normalen und diabetischen organismus. Klin. Wochenschr., vol. 5, page 2100, 1926.

organs especially characteristic of immaturity, like the thymus, develop glycogenolytic functions earlier than adult tissues. This is not true, however, of the brain.

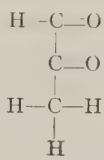
The Fate of Glucose.— After the formation of glucose from the stored glycogen, or after it has been ingested directly, it is utilized by the tissues, especially muscle tissue, where it participates in the production of heat and muscle energy. We have spoken of the rôle glucose plays in muscle contraction, and here we may add that glucose pursues one of two different courses: (1) Anaërobic metabolism; glucose is converted into lactic acid without the aid of oxygen admitted from without, according to the following reactions:



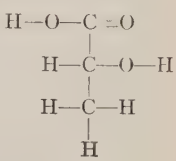
Glucose.



Two glyceric aldehydes.



Methylglyoxal.



Lactic acid.

(2) Aërobic metabolism, during which glucose is oxidized. It is oxidized as a hexose and not as lactic acid, or any other product of glucose, as far as can be determined. Insulin is necessary for the oxidation of the sugar. There is no infallible evidence that a special reactive form of glucose is essential to the combination. We know that artificially prepared solutions of glucose, like that in Locke's solution[1] are utilized to a greater extent by tissues such as the beating heart when in the presence of insulin, perfused through the isolated heart along with the glucose solution, than when insulin is absent. Consequently, we know that we are dealing with but two factors, glucose and insulin.[2] How does insulin affect glucose to render it utilizable? Ordinary glucose is stable and its formula shows that its reactive radicle, the aldehyde radicle, is bound up in a ring, whereas if glucose is active, we must assume that the aldehyde group is free or easily made free. The stable sugars are known as oxidic sugars, and

[1] Page 401.

[2] The possibility that a phosphate-glucid compound is necessary for sugar utilization has been suggested, and it is interesting to observe the correlations between phosphates in the blood, glucose, and insulin mentioned on page 513. Evidence, however, is lacking that the phosphate is necessary in the utilization of glucose.

the labile ones, aldehydic, the former term referring to the oxygen linkage.[1] A possible form of glucose available for oxidation is shown in model form in the photograph, Fig. 88, page 180. Its aldehydic character is seen in the carbon-atom numbered 1, at the extreme right of the group. The oxygen ring is attached to carbons 2 and 5. Whenever the oxygen ring does not include carbon 5, the substance is called a "gamma sugar." It is one in which the oxygen linkage is other than a butylene ring. There is no indisputable evidence that glucose metabolizes as a gamma sugar, but all evidence points to the fact that it is burned by virtue of its aldehydic properties. That insulin does something to render glucose more readily utilizable suggests that it may cause the conversion of a stable sugar (oxidic) to a labile one (aldehydic), but no proof exists. Winter and Smith[2] have reported observations supporting this theory, but other investigators[3] have failed to corroborate their findings in this regard.

To the question *What becomes of glucose when it is oxidized?* we may say that the oxidation of any aldehyde produces an acid. In the case of glucose there are three possibilities, according to the amount of oxygen or of oxidizing power: Gluconic, glucuronic, and saccharic acids. Gluconic acid is the only one of the three incapable of forming an oxidic acid (lactone) and it is probable that the first product of the oxidation is this acid. Further than this we are ignorant at the present time. Insulin causes the disappearance of glucose from the blood, but there is not a corresponding elimination of CO_2 and utilization of oxygen in the normal animal. Glycogen is not stored. Glucose is not converted into fat.[4] Glucose may be polymerized to form sugars of the nature of caramel. The diabetic can utilize, to some extent, scorched sugars, caramel, etc.

After excessive muscle exercise, as during convulsions, glucose disappears from the blood and lactic acid accumulates. However,

[1] Page 182.

[2] Winter, L. B., and Smith, W. (Cambridge, England), Jour. Physiology, vol. 58, p. 100, 1922.

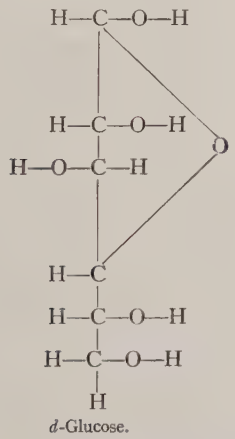
[3] Hewitt, J. A., and Pryde, J. (Kings College, London), Biochem. Jour., vol. 14, p. 395, 1920. Van Creveld, S. (Groningen, Holland), Biochem. Jour., vol. 17, p. 860, 1923. Visscher, M. B. (University of Minnesota), Amer. Jour. Physiol., vol. 68, p. 135, 1924. Denis, W., and Hume, H. V., Jour. Biol. Chem., vol. 60, p. 603, 1924. Also Jour. Biol. Chem., vol. 59, p. 457, 1924. Recently Pryde and others have obtained evidence that the reactive form of glucose is "none other than the butylene oxidic form which has for years been ascribed to the stable sugar." Gamma lactone is "gamma sugar."

[4] Dudley, H. W., and Marrian, G. F. (National Institute for Medical Research, Hamsted, England), Biochem. Jour., vol. 17, p. 435, 1923. This statement is not contradicted by that on page 524. In the present case we mean glucose is not converted into fat *under the influence of insulin.*

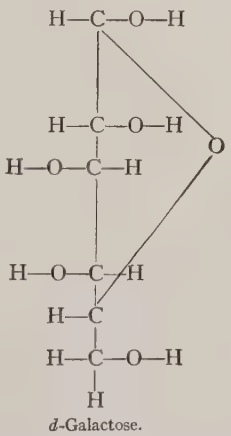
insulin does not affect the concentration of lactic acid. Epinephrin does increase the lactic acid in the blood.[1] It would appear that the lactic acid is a part of some reaction not immediately related to glucose utilization. Nor does this acid arise from a cleaving of a lactic acid-phosphoric compound, which has been assumed by Embden[2] to play a rôle in glucid metabolism.[3]

Utilization of Glucose in Other Ways.—Besides the utilization of glucose in energy transformations, such as we have discussed above, it participates in structural matters. Glucose forms a part of the nucleoprotid of the nucleus of the cells; it enters into the formation of scleroprotids, like mucin, and it may be a factor in many other structures the chemistry of which is not fully understood.

The Utilization of Sugars Other than Glucose.—On page 171 we have discussed the special chemistry of fructose, which is being used to greater extent in human dietaries. Fructose does not form a part of the body as glucose and galactose do. Fructose, however, as we have said, is convertible into glucose, and through glucose to galactose. Galactose is used by the young of both sexes in the structure of the nervous system, in which we find galactose as galactoside. In the male galactose does not appear in any other tissue, but in the female it is also found in the mammary gland. Galactose is formed from glucose and becomes united into a diglucid, the galactoside, lactose:



d-Glucose.



d-Galactose.

[1] Cori, C. F. (Institute for Malignant Diseases, Buffalo, N. Y.), Jour. Biol. Chem., vol. 63, p. lii (Proceedings), 1925. This author has recently reported a temporary storage of glycogen when insulin is administered.

[2] Embden, G., German physiologist.

[3] Morgulis, S., and Barkus, O. (University of Nebraska College of Medicine, Omaha), Jour. Biol. Chem., vol. 63, p. lxviii (Proc.), 1925.

The mechanism of the transformation is not known. When the mammary glands are lactating, the feeding of glucose leads to galactosuria or to lactosuria.

The utilization of the sugars is not a matter of their relative reducing powers. Arranged in the order of reducing power, the common sugars are:[1]

		Units as standard.
Glucose	amount reduced in six minutes	= 100
Levulose	" " " "	= 90
Galactose	" " " "	= 77
Mannose	" " " "	= 55
Lactose	" " " "	= 45
Maltose	" " " "	= 40

This is not the order of these sugars in metabolism.

The pentose, ribose, forms a part of the free mononucleotids, guanylic and inosinic acids.[2] It does not occur in the cell nuclei. This pentose is the one found in the urine in pentosuria,[3] although some identify it as the inactive *d-l*-arabinose and others as *l*-xylose. Lactose derived from the food is probably not directly transformed into the lactose of the mammary gland on account of its low absorbability and consequent hydrolysis. Lactose is of such low absorbability that it has the cathartic action mentioned earlier in this book.[4] Galactose is not recognizable in the circulation except in the portal system after a considerable ingestion of lactose or galactose.[5]

Summary.—1. All glucids are utilized as glucose.

2. Glucose may be converted into other glucids, like galactose, and utilized as such.

3. Glucids taken into the alimentary canal are absorbed; or hydrolyzed and then absorbed.

4. They are carried as monoglucids to the liver, in which organ they are converted into glucose before they enter the general circulation.

[1] The table is from Rowe, A. W., and Wiener, B. S. (Evans Memorial Hospital, Boston, U. S. A.), Jour. Biol. Chem., vol. 63, p. lxxiii (Proceedings), 1925. The figures are for the Folin-Wu method (Chapter XVI); Lewis-Benedict analyses run higher as a rule.

[2] Page 339.

[3] Chapter XV. Berglund (cited on page 505) finds that the amount of pentose in the urine is independent of the amount injected into the veins in an experimental animal.

[4] Page 491.

[5] Rowe (page 505) has recently proposed a sugar tolerance test based on the peculiar metabolism of galactose. The average human male tolerates 30 gs. of galactose administered by mouth and the female 40 gs. without galactosuria. Tolerance is defined as the smallest amount of galactose which will produce a detectable quantity of galactose in the urine.

5. Glucids other than glucose are converted into it before entering the general circulation.

6. Muscle glycogen is formed from glucose.

7. Other glucids, like galactose, are formed from glucose.

8. The steps in glucid oxidation are not known.

9. Glucose is used in a dual manner in muscle action: (*a*) Some of the molecules of glucose are converted into lactic acid which increases the permeability of the muscle for water, etc. This acid is burned to produce the heat necessary for the reaction. (*b*) The remaining molecules of lactic acid are reconverted into glycogen.

10. Glucose may be converted into a hexosephosphate before utilization.

11. Insulin causes glucose to disappear from the blood of a normal subject.

12. The fat of this glucose is unknown.

13. Insulin causes the utilization of glucose and storage of glycogen in a diabetic animal.

14. The glucids are utilized not only in the production of mechanical and heat energy, but also enter into the structure of the organism (brain, mucus, etc.).

THE UTILIZATION OF THE LIPIDS

The finely dispersed fat delivered to the blood-stream from the thoracic duct exists in spheres about 0.001 mm. in diameter (Fig. 157). About 50 per cent. of these droplets is taken into the red and white corpuscles, not as neutral fat, but as phosphorized fat, or prospholipid, like lecithin; or some is made into a cholesterol ester. Lecithins occur to a negligible extent in the plasma of the blood. If there is an increase of blood lipids beyond the usual amounts (0.6 g. per 100 mls. whole blood), then cholesterol begins to increase (from a normal of 0.16 g. per cent.) in order to compensate for the increase of neutral and phosphorized fat; for cholesterol and lecithins act antagonistically in the body, cholesterol holding water, while lecithin tends to cause dehydration of the tissue elements. Cholesterol protects the erythrocytes from such action. Moreover, when the fat content of blood is high, cholesterol increases because the cholesterol esters have been saponified, freeing fatty acids, which become synthesized into neutral fats, and the cholesterol, being in like manner free, accumulates in the blood. A small content of free fatty acids accompanies the blood lipids. Some of these may become soaps, which cannot exist to any great degree in blood, owing to their

toxicity. Others, as we have said, become cholesterol esters which normally comprise about one-third of the total cholesterol content of the blood. Bloor has recently determined that cholesterol esters play a more important part in fat transportation than we have hitherto believed. The unsaturated fatty acids are practically all carried as such esters.[1] This, then, is the condition of the lipids as they are distributed throughout the body in the blood.

Fig. 163.—P. A. Shaffer, Professor of Biochemistry, Washington University, Saint Louis, Missouri. Contributor to the biochemistry of sugar, ketogenesis, etc., with methods for determination of various substances of biochemical importance.

Passage of Fat from Blood to Tissue.—On page 494 it has been stated that inasmuch as lipases adequate to account for hydrolysis and subsequent synthesis of fats as they leave the blood and enter the organs, do not exist in the tissues, it is necessary to assume that the transfer from blood to tissue is effected by a movement of fat, as such, and not as hydrolysis products, glycerol and fatty acids which become resynthesized in the organ. We must remember that lymph intermediates between blood and tissue and that lymph, throughout the body, transports fat.

[1] In dog plasma unsaturated acids are present to the extent of 70 per cent., nearly all of which is cholesterol ester.

The Character of Tissue Fat.—The fat of tissues may be subdivided into two great classes: (1) Dépot fat, characterized by the high content of saturated fats (tripalmitid and tristearid); such fat occurs in the omentum, in the "suet" of the kidney and heart, and in other storage regions. (2) Tissue fat, characterized by its high content of unsaturated lipids, like lecithin; such fat occurs in the liver especially, but also in the tissues of the kidney, heart, etc.

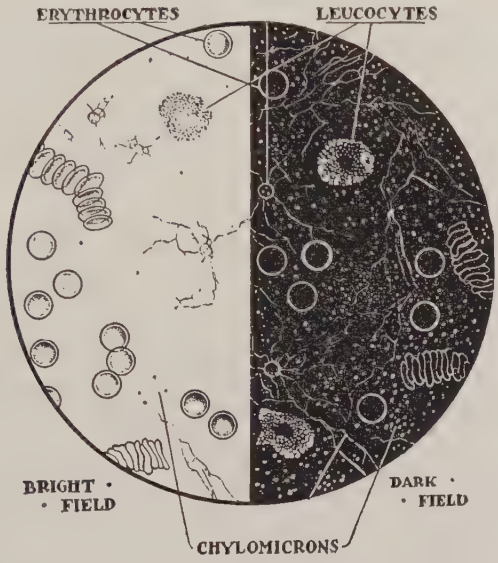


Fig. 164.—Fat "dust" as seen by the ultramicroscope (see Fig. 58). (After Gage, Amer. Jour. Anat., vol. 34, No. 1, September 15, 1924.)

Liver Fat.—It is well known that liver contains fluid fat (like cod-liver oil). Such lipid is characterized by its high iodin number[1] and its ability to give up unsaturated fatty acids on hydrolysis. These acids are capable of hydrogenation by the addition of hydrogen in the presence of a catalyzer. They are more soluble in water if the unsaturated carbon atoms are satisfied by hydroxyls during hydrogenation. The content of phospholipids having unsaturated acids, like oleïc, is high in the liver; this is indicated by the iodin absorption value:

	Iodin number.
Human adipose fat	65
Human liver fat	110

When there is an abnormal acquisition of fat by the liver, storage fat is laid down either by fatty "degeneration"[2] or by infiltration.

[1] Page 205.

[2] Page 380. The origin of fat from other kinds of substances is still in dispute.

There is a progressive decrease in iodin absorption, indicating that such fat is not the fat of normal metabolism of the liver:

	Higher fatty acids.	Iodin number.
Normal liver	12.1	116
Early toxic jaundice	15.6	109
Bronchopneumonia	54.6	71
Trauma	66.3	63

One of the functions of the liver seems to be that of desaturating the fats and of transforming them into phosphorized fats, the lecithins and other phospholipids, which are more labile than neutral fats of saturated fatty acids, such as are found in the dépots. The reason for this desaturating is probably that the unsaturated fats pass through membranes more readily than the saturated forms; it may have something to do with the melting-point, which is lowered by desaturation. These phospholipids, once formed in the liver, may be stored for a period, but we know that the total lipid content of the liver does not change to any great extent and doubtless it acts as a manufacturing plant where fatty acids are rendered more labile, preparatory for use in the body elsewhere and that there is a constant influx of lipids and outflow of phospholipids, while the total content of the liver remains fairly constant. Since not only the liver, but other organs, like the kidney, have a high content of unsaturated acids in the form of phospholipids, it is probable that this desaturating function is shared by various, if not all, tissues.

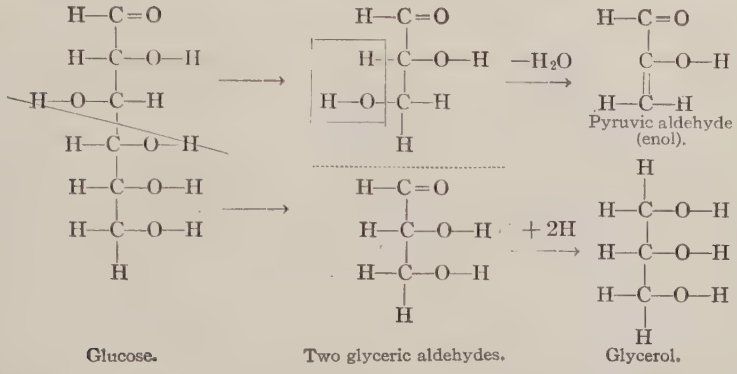
Fat from Glucids.—Since 1883 we have known definitely that 19.5 per cent. of glucids may be converted into fat, whereas only half that amount of fat can be converted into glucid. In such a process (fat to glucid) about 4.7 per cent. of the heat value is lost.[1] Such a conversion would involve a loss of oxygen from the molecule of the glucid,[2] and when this occurs in the organism the Respiratory Quotient,[3] $\frac{CO_2}{O_2}$, may be above that for a glucid diet in which the ratio is unity. This is due obviously to the reduction of the glucid and a relatively greater amount of exhaled CO_2 compared with the oxygen consumed. This reduction must be accompanied by condensation, because the smallest of the fatty acids found in any of the fats contains 16 carbons, while glucose contains but 6.

[1] Fat from glucid is composed largely of higher melting-point fats (stearid and palmitid).

[2] Compare the oxygen content of glucose ($C_6H_{12}O_6$) with that of a fatty acid ($C_{16}H_{32}O_2$).

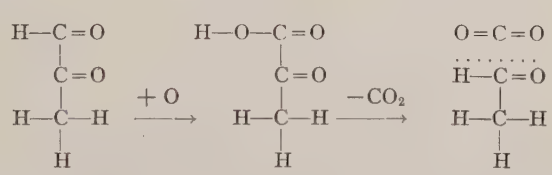
[3] Page 616.

The derivation of fat from glucids is represented by the following reactions. Unfortunately, there are postulates which have not been verified by actual observation, but each step can be justified by investigations of a number of workers. Beginning with glucose:



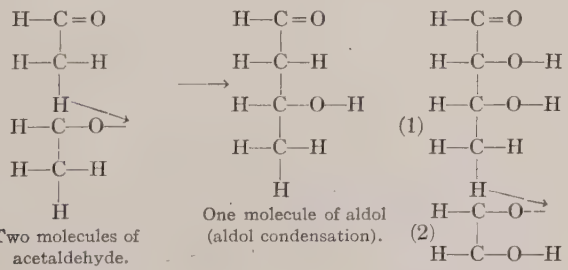
Glucose. Two glyceric aldehydes. Glycerol.

Glycerol is obtained by the reduction of glyceric aldehyde; the reaction is that of the Cannizzaro (page 249). The remaining portion of the aldose, glucose (upper portion) follows the Cannizzaro reaction in becoming oxidized to an acid, pyruvic acid, via enolketo intermediate forms:



Pyruvic aldehyde (keto). Pyruvic acid. CO_2 and acetaldehyde.

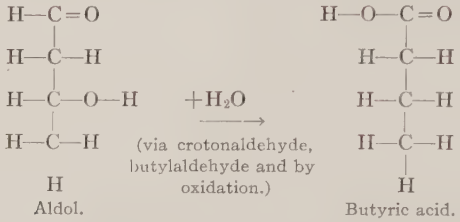
Thenceforward, acetaldehyde undergoes condensation which represents the first of a series of such changes:



Two molecules of acetaldehyde. One molecule of aldol (aldol condensation). Two molecules of aldol condensing to form an 8-carbon acid. (Upper part of 2 only shown.)

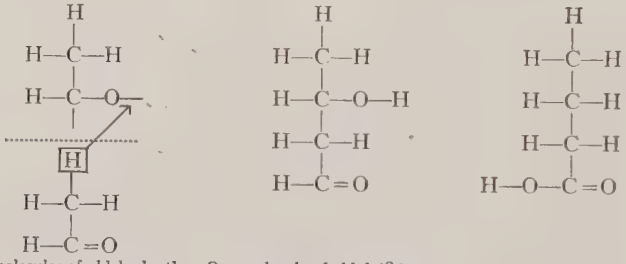
The octylic acid (8-carbon) then may condense to form a 16 carbon acid; or, since it is probable that an 18-carbon acid is fundamental in

metabolism (oleïc; stearic), acetaldehyde and aldol may condense to form such a compound. Still another path may be followed by the conversion of aldol (β-hydroxy-butyric aldehyde) to butyric acid:



Butyric acid occurs in faulty glucid and fat metabolism as either the hydroxy (β-hydroxy-butyric acid) or keto-acid, aceto-acetic (di-acetic).

(4) The aldehyde thus produced is condensed in aldol condensation to butyric acid; two aldehydes are involved:



Butyric acid or any other 4-carbon acid cannot be burned by a diabetic, especially when the beta-carbon atom bears an hydroxyl, as in the case of β-hydroxy-butyric acid, or an oxygen in the carbonyl form, as in aceto-acetic acid. However, the normal animal is capable of causing the β-carbon to become oxidized, thus giving rise to two molecules of acetic acid.[1] This is the normal course of oxidation of the fats and is known as beta-oxidation. The fatty acid portion of the amino-acids is metabolized in the same way.

If, now, there is a synthesis of lactic acid into a fatty acid of the higher series, like stearic, palmitic, or oleïc, a larger number of carbons must be involved, which means that more than two aldehyde molecules are condensed. Thus, if four were condensed, an octylic acid, like caprylic acid having 8 carbons, is formed. Eight molecules of

[1] Shaffer, P. A., Jour. Biol. Chem., vol. 61, p. 585, 1924, believes that the reason why the diabetic animal does not oxidize such substances is that they occur in larger amounts than there is "ketolytic" substance (page 534) present to cause their further oxidation to CO_2 and H_2O.

acetaldehyde condense into a 16-carbon chain, to which stearic acid belongs. In some such manner the fatty acids of the fats are formed from glucid. We shall see later[1] that such conversions do not involve much change in energy, whereas the oxidation of the fats leads to the greatest proportional production of energy in the body.

Fat from Protids.—No direct proof exists of the origin of fat from protid. The classical and oft-quoted experiments of Weinland[2] who analyzed fly-larvæ (maggots) before and after feeding protid and found an increase of fat, while never refuted, have never been confirmed since modern analytical methods have been devised. Kumagawa[3] made a direct experiment upon dogs as follows: Two dogs from the same litter were selected and both were starved for twenty-four days. At the end of this time the control was killed and the whole body analyzed for fat. The second dog was fed plentifully with lean horse meat for forty-nine days, at the rate of about 1 kilo per day. This increased his weight 3.92 kilos. Analysis of the fat of this animal showed an increase of 968 gs. The food (49 kilos of horse meat) contained 1084 gs. of fat, an amount abundantly able to account for the increase in weight of the animal, even without calling in the 356 gs. of glycogen of the food, which might have contributed to the fat accumulating during the feeding period. There was no indication that protid from food had entered into the formation of the fat of the dog. Indirect determinations, however, by estimating the accrued fat in terms of the carbon retained after a period of feeding seem to indicate that 40 per cent. of the ingested protid capable of conversion to glucose was transformed into fat. Actual experimentation by Lusk[4] leaves 33 per cent. of glucose not accounted for after estimating the amount of glycogen stored.

The chief interest in the question concerning the origin of fats from protids, for the medical student, lies in its bearing upon degenerative changes of organs, or fatty degeneration, the liver, kidney, and other organs show. Under certain conditions, like phosphorus poisoning or acute yellow atrophy, these organs degenerate. Apparently, when examined microscopically, increased fat has occurred. It is definitely known that this is a process either of mobilization of minute fat "dust" normally present in the cells, into larger aggre-

[1] Page 570.

[2] Zeitschr. für Biol., vol. 51, page 197, 1908.

[3] Kumagawa, Muneo, University of Tokio, Japan.

[4] Atkinson, H. V., Rapport, D., and Lusk, G., Jour. Biol. Chem., vol. 53, p. 155, 1922.

gates of fat, a process known as phanerosis;[1] or else of infiltration of fat outside the tissue in question. If analysis be made of the fat content of normal tissue in which fat is evident macroscopically, and of similar but abnormal tissue exhibiting fat, in the majority of cases there is no actual increase in the total amount of fat in the second instance. In a few cases more fat is found in the abnormal tissue than in the normal control. This is explained by transfer of fat from without, or fatty infiltration.[2] In making such studies, it is necessary to exercise great care in obtaining all of the fat in the tissue, for some of it is held in chemical union with other substances, as in associations of fatty acids and amino-acids; this must be freed by digesting the tissue with pepsin. As a rule, fatty degeneration involves less fat rather than more. By studying the chemical characteristics of the fat found in this condition, one can determine whether much or any fat has entered the tissue from without. We have already observed[3] that as abnormal fat metabolism appears in a tissue the fat becomes more saturated, as indicated by the fall of iodin number. Somewhat similar infiltration of tissue by fat or fatty components is found in cadavers lying in moist places. Here the protid structure disappears and is replaced by adipocere,[4] but a considerable loss of weight accompanies this transformation. The adipocere is known to arise wholly from the lipids of the body, the protids undergoing autolysis and disappearing under the action of bacteria. It is possible that bacteria and other low forms of life are capable of synthesizing lipids from protids. In fasting, it is the saturated, "hard," higher melting-point fats which disappear. Even at death, the organ (unsaturated) fat is but little reduced in amount. The organ fat is a part of the vital mechanism. This is emphasized in the case of the central nervous system (page 400).

Lipidtemns.—Free fatty acids occur under certain conditions, especially in diseased tissues. They are frequently found as salts of calcium (calcium oleate, etc.) and the less fluid fatty acids, stearic and palmitic are sometimes found as "margarin," or mechanical mixtures of these acids —the crystals being the resultant of the separate crystallization of these fatty acids and not a specific crystal of

[1] Greek *phaino*, to brighten, *phaneros*, evident or manifest; hence fat which becomes manifest or seen. Compare tryptophan. Introduction of bacterial toxins, such as that of bacteria, will cause phanerosis.

[2] This is true in phosphorus poisoning and also in fasting; in both cases, a mobilization of depôt fat occurs and this fat is transferred to the liver; but the resulting fat is of higher melting-point than normal liver fat.

[3] Page 524.

[4] Latin *adeps*, lard, and *cera*, wax; pronounced "ad'i-po-sere."

a chemical compound to these two acids. Cholesterol esters of these fatty acids in which cholesterol is the alcohol and palmitic, stearic, or oleïc acid is the acid, occur in degenerative tissues such as tumors. Cholesterol itself is found in similar tissues and probably serves to protect the cells so far as possible from destruction while the degenerative changes are taking place. It is difficult to believe that it is simply present as a by-product of metabolism, for we know of its participation in protective processes in the body. Perhaps another adaptation to the destructive changes in "amyloid degenerations"[1] is the development of the substance known by that name, amyloid, known to be related to, but not identical with, nucleoprotids. It has a low content of phosphorus which is not true of nucleoprotids and, besides, it contains a relatively high amount of sulphur, suggesting that it is related to chondroitin sulphuric acid;[2] furthermore, the suggestion has been made that amyloid is a conjugation product of this acid with a simple protid, like a histon,[3] in a manner similar to that in which nucleoprotid is conjugated as a salt of nucleic acid, with a protid, protamin, or histon. This is rendered likely by a study of the amino-acid content of amyloid. It will be recalled that the simple protids, like protamin or histon, consist largely of diamino-acids, arginin and lysin. Amyloid contains a large proportion of these diamino-acids, as shown by the following table; for comparison, the amino-acid content of a histon, thymus histon, is given (after Wells, H. G.):

	Amyloid, grams per 100 grams substance.	Thymus histon, grams per 100 grams substance.
Glycin	0.8	0.5
Leucin	22.8	11.8
Glutamic acid	3.8	5.2
Tyrosin	4.0	5.2
Prolin	3.1	1.5
Arginin	13.9	14.5
Lysin	11.6	7.7

Amyloid, then, is probably a conjugation product, the base being a simple protid as in nucleoprotid, but the acid, sulphuric acid and not nucleic acid. There are no fat relations, therefore, in amyloid.

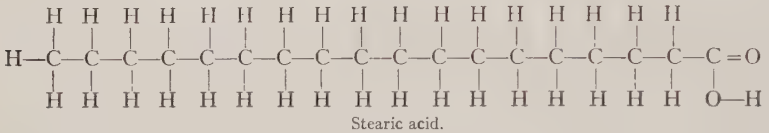
The Oxidation of Fats.—Fat is a typical heat-producing substance. The fats contribute more heat units, or calories, than any

[1] The term "amyloid" (Greek *amylon*, starch) was used by the father of pathology, Rudolph Virchow, to apply to a caseogenous or lard-like degeneration occurring in kidneys and other organs because he thought this substance was of glucid nature, belonging to the celluloses.

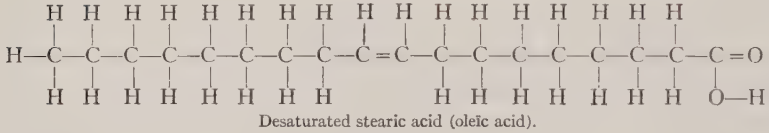
[2] Page 354.

[3] Page 326.

other food. The principle of oxidation of the fats has been referred to[1] as consisting of the oxidation of the beta, or antepenultimate carbon atom of the chain. However, before this process begins, the fat is hydrolyzed to glycerol and fatty acids and oxidation involves the separate behavior of each of these substances. The glycerol behaves as a glucid,[2] while the fatty acid is desaturated in the liver; the chain is weakened in the middle by the withdrawal of two or more hydrogen atoms, thus:

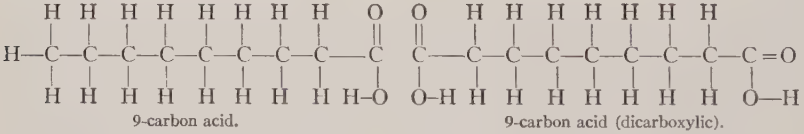


Stearic acid.



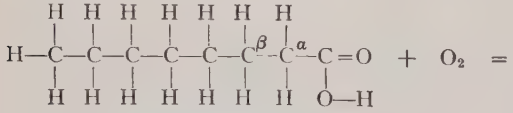
Desaturated stearic acid (oleïc acid).

Then there is an oxidation of the unsaturated carbon atoms and a rupture of the chain:

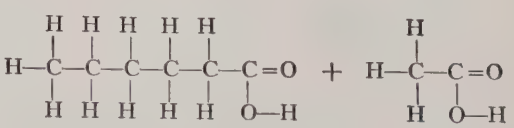


9-carbon acid. 9-carbon acid (dicarboxylic).

These odd-number carbon chains are then converted by decarboxylation to an even number chain, after which there begins an oxidation of the β-carbons atoms in each case, giving rise to acetic acid and to an acid having two carbon atoms fewer than before.



8-carbon acid (like caprylic). Oxidized.



6-carbon acid. Acetic acid.

[1] The theory of Knoop. Page 204.

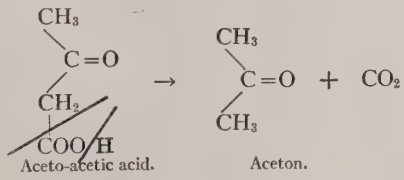
[2] The method of oxidation of the sugars is given on pages 156 and 176.

Smith[1] has recently given critical attention to the theory just described. He finds that azelaïc acid (page 202), a dicarboxylic acid commonly obtained from unsaturated acids is burned in a normal animal only to the extent of 60 per cent. On the other hand, pelargonic acid, obtained from ordinary oleïc acid is completely oxidized. These experiments are interpreted as not corroborating the theory of Leathes. In all cases this process of cleaving off a molecule of acetic acid, oxidizing the residue, then cleaving off another acetic molecule, etc., progresses until the last 4 carbons, $CH_2.CH_2.CH_2.COOH$ are reached, which is equivalent to a butyric acid molecule. In the diabetic, oxidation may go no farther, but, normally, this group of butyric carbons in the form of β-keto-butyric acid (commonly called aceto-acetic acid, or diacetic acid) is metabolized ultimately to CO_2 and H_2O. Exactly how the 4-carbon aceto-acetic acid becomes oxidized is not known, but it is probable that it undergoes conversion to two compounds, acetic aldehyde, CH_3CHO, glyoxylic acid, CHO.COOH, or formic acid, H.COOH, whence it becomes by oxidation, CO_2 and H_2O. In acidosis, aceto-acetic acid is related to aceton, $(CH_3)_2C{=}O$, which is aceto-acetic acid minus CO_2.[2] This does not necessarily represent the relation in acidosis, but probably it is the procedure, since the relation goes on spontaneously in alkaline medium, or more quickly with an oxidizing agent like H_2O_2. In some unknown way glucose reacts in such an equation as that representing the conversion of aceto-acetic acid into aceton, favoring the reaction; in this case, the function of glucose, or its derivative, is called ketolytic, since it causes the keton-acid, aceto-acetic acid, to break up. Ketolysis and related processes will be discussed in detail later (page 534).

Besides β-keto-butyric acid, another 4-carbon acid, built upon butyric acid occurs in acidosis; this acid is β-hydroxy-butyric acid,

[1] Smith, H. G. (University of Rochester, N. Y.), Journ. Biol. Chem., vol. 67, page xlvii, (Proc.), 1926.

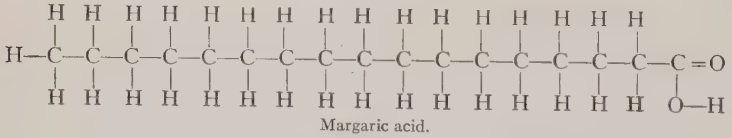
[2] The relations are as follows:



Aceto-acetic acid. Aceton.

$CH_3.CHOH.CH_2.COOH$.[1] Insulin in the presence of glucose causes the 4-carbon acids to undergo normal metabolism, or what amounts to this.[2] Even in the test-tube, the reaction may be shown if the reaction is alkaline; glucose, on being oxidized,[3] causes the oxidation of aceto-acetic acid. What the substance is, produced by some unknown action upon glucose and which permits the burning of the ketoses[4] in an apparently normal manner, is not known, but in all probability, a 6-carbon compound is responsible for the reaction. Since caramel, roasted starch, and similar polymerized glucids are said to be utilizable by the diabetic organism, causing either the suppression or disappearance of the keto-substances, it may be some polymere of glucose that acts as a ketolytic substance.[5] We have seen[6] that tissues cleave hexosephosphate; the resulting glucose, split from the phosphoric acid, may be antiketogenic. We know that glucose forms hexosephosphate and that the hexosephosphate is cleaved in the blood.[7]

Carbon Atom Number and Fat Utilization.—It has already been observed that the normal animal is capable of utilizing 4-carbon compounds left after β-oxidation has progressed for some time, while the diabetic animal cannot. All natural fats are composed of even-numbered carbon atoms. However, the diabetic as well as the normal animal can utilize fats which have odd-carbon fatty acids like margaric, $CH_3(CH_2)_{15}COOH$ or $C_{17}H_{34}O_2$:



Margaric acid.

Such acids do not occur in nature, but are made in the laboratory.[8] Ringer found that odd-carbon fats and fatty acids are utilized by the

[1] β-hydroxybutyric acid is probably derived from β-keto-butyric acid and represents a by-product.

[2] See Killian, J. A. (New York Post-Graduate Medical School), Proc. Soc. Exp. Biol. and Med., vol. 21, p. 22, 1923.

[3] Or otherwise changed.

[4] Rosenfeld: "Fat burns in the fire of the carbohydrate." Ascher-Spiro, Ergebnisse, I, Biochemie, 1902.

[5] A substance which prevents the formation of keto-acids and ketones is known as an antiketogenic substance.

[6] Page 176.

[7] Page 176; also consult the paper of Chaikoff, Macleod, and Markowitz, page 511.

[8] The commercial product is known by the trade name of "intarvin" and is made by the Bergman Laboratories, New York. It is a fat composed of glycerol and 3 mole-

diabetic, owing to the fact that β-oxidation carries the last group to a 3-carbon and not to a 4-carbon chain, as in the case of the naturally occurring fats and acids. Thus, propionic acid, $CH_3.CH_2.COOH$ is formed and is capable of being converted into glucose. For this reason, glucose appears in the urine of a subject being fed one of the odd-carbon substances.

The Antiketogenic Action of Odd-carbon Compounds.—At the present time it is difficult to make definite statements regarding the utility of odd-carbon compounds in the organism, for while animal experimentation and observation on human subjects by some observers[1] show absorption of the fat (intarvin) to the extent of 95 per cent.; non-toxicity; ketolytic and antiketogenic properties; appeasing of hunger and prevention of loss of weight; on the other hand, other observers[2] fail to find ketolytic effects. Sevringhaus fed intarvin to two men up to 100 gs. per day without lessening the acidosis induced by fasting. Since glucose is not affected by these odd-carbon fats, so that this sugar exerts its ketolytic and antiketogenic action, any favorable effect from odd-carbon fats must be sought in the reduction of acidity of the body and to a utilization neither of the natural even-carbon fats nor of glucose. Glucose, indirectly, may be affected, since by lowering the acidity, glucose is more readily utilized.

The Quantitative Relations between Ketogenic and Antiketogenic Substances.—The hospital diet for diabetics is based in part upon the quantitative relation between those foods which do not produce keto-acids but rather inhibit their production, and foods that produce such acids. While aceto-acetic acid is usually formed from fats, we know that certain amino-acids, leucin, phenylalanin, and tyrosin can also form it. On the other hand, glucids are antiketogenic. Shaffer[3] has studied the problem in detail.

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cules of margaric acid. One method of preparing it is to boil cetyl cyanid, obtained by heating cetyl alcohol with KCN, with KOH. The fact that odd-carbon fats and acids, like valeric, $CH_3(CH_2)_3COOH$, and nonylic acids, $CH_3(CH_2)_5COOH$, are capable of forming glucose was recognized by A. I. Ringer (then of the University of Pennsylvania, now of Montefiore Hospital, New York). See his papers in the Jour. Biol. Chem., vol. 12, p. 511, 1918, and later.

[1] Heft, H. L., Kahn, M., and Gies, W. J. (Columbia University and Beth Israel Hospital, New York), Jour. Biol. Chem., vol. 63, p. lxvii (Proceedings), 1925. Also Lundin, A., Jour. Metabol. Research, vol. 4, p. 151, 1923.

[2] Sevringhaus, E. L. (Wisconsin), Jour. Biol. Chem., vol. 59, p. xlix (Proceedings), 1924.

[3] Shaffer, P. A. (Washington University, St. Louis, Mo.), Jour. Biol. Chem., vol. 47, p. 433, 1921, and later papers in the same journal.

Antiketogenesis.[1]—Shaffer found that when the keto-acid, aceto-acetic acid, which appears in the urine of patients with acidosis, is boiled with glucose in an alkaline solution in the presence of hydrogen peroxid as an oxidizing agent, about one-half of the acid disappears. In the absence of glucose about one-third as much of the keto-acid is destroyed. Fructose and glycerol also act in the process of destruction of the keto-acid. Other substances, such as lactic acid, which are known to be products of glucose disintegration, do not act as ketolytic[2] substances. In this test-tube experiment of Shaffer glucose is burned at the same time that the keto-acid is destroyed.

It is known that when glucose is given to a person who excretes aceto-acetic acid, an antiketogenic or ketolytic action inhibits the further excretion of the acid if the glucose is utilized. By analogy with the test-tube experiment of Shaffer just described, glucose is burned and thereby the keto-acid is destroyed (ketolysis); moreover, the action may involve the oxidation of the substances which produce the keto-acid (fat, principally) before the acid is formed (antiketogenesis). Three hypotheses have been offered for this protective action of glucose against the formation of the keto-acid or for the destruction of the acid once formed:

(1) A keton-glucose compound necessary for the burning of these two substances does not form and therefore they accumulate.

(2) Certain products are formed when glucose is oxidized that cause the burning of the keto-substance.

(3) Glucose joins with the keto-substance having 4 carbon atoms to produce a compound that has such a configuration that a keto-substance does not form during oxidation. The compound of glucose and aceto-acetic acid has 10 carbon atoms (glucose 6 and the keto-acid 4, making 10). This 10-carbon compound is cleaved into two halves each, with 5 carbon atoms, from which a molecule of acetaldehyde (2 carbon atoms) and one of propylaldehyde (3 carbons) are produced. These compounds can be burned to CO_2 and H_2O.

None of these theories is adequate and it is not known at the present time what rôle glucose plays.

[1] Greek *anti*, against; *keto* refers to the keto-acid, aceto-acetic, and to the keton, aceton, and *gen*, to produce. A substance which prevents the formation of keto-substances. Dakin first performed the experiment which Shaffer used as a basis of his computations. Likewise, Shaffer's data was drawn in part from the work of Woodyatt (page 768). These historical notes do not detract from Shaffer's credit for he was a pioneer in this line of investigation.

[2] Greek *keto*, and *lysis*, to loosen. The term is applied to substances which destroy ketones.

Calculations of Keto-antiketogenic Ratios.—It is possible, by carefully analyzing the factors, to place this relation upon a fairly definite, quantitative basis. Fats give rise to the larger amount of aceto-acetic acid: 1 g. fat produces 0.35 g. aceto-acetic acid.[1]

Some amino-acids give rise to keto-substances:

	G. mol. aceto-acetic acid.
1 g. leucin gives	0.109
1 g. phenylalanin	0.027
1 g. tyrosin	0.024
Total for 16.18 gs. nitrogen[1]	0.160
1 g. nitrogen, 1/16.18 of 0.160	0.010

[1] The amount of nitrogen in 100 gs. protid.

The total ketogenic value of the foods, then, may be determined by estimating the amount of fat and of food-nitrogen administered.

Turning to the *antiketogenic substances*, we know that glucose is utilized as a whole to produce the antiketogenic substance and we may assume that each molecule of glucose produces one of the antiketogenic substances. The molecular weight of glucose is 180; 1 g of glucose $\simeq$ 1/180 g. mol. antiketogenic substance, that is, 0.00556 g. mol. Then:

1 g. glucose produces 0.00556 g. mol. antiketogenic substance.

Again, we have shown that certain amino-acids can produce glucose. Corresponding to 1 g. nitrogen, there are 3.6 gs. glucose. Therefore, 1/180 of 3.6 or 0.020 g. mol. of glucose corresponds to 1 g. nitrogen. By determining the total nitrogen of the urine, we are able to tell the amount of antiketogenic substance, expressed as glucose, produced in the body during the time the urine was collected.

1 g. urinary nitrogen $\simeq$ 0.02 g. antiketogenic glucid.

The keto-antiketogenic ratio $\left(\frac{K}{A}\right)$ is therefore:

$$\frac{\text{Sum of keto-substance from fat and that from protid (urine nitrogen)}}{\text{Sum of antiketogenic substance from glucose and from protid (urine n.}^{2}\text{)}}$$

[1] This is based upon the theoretically possible amount of β-hydroxy-butyric acid which can be obtained from mixed body fat; 36 gs. may be produced from 100 gs. of fat. The average molecular weight of the three fats found in body fat, stearid, palmitid, and oleïd, is 874. Three molecules of aceto-acetic acid are derived from 1 gram of fat. Hence 1/874 gram-molecule of fat capable of producing aceto-acetic acid times 3 gives, in decimal fraction, 0.003432 gram-molecule, the amount of aceto-acetic acid 1 gram of fat will give. The molecular weight of aceto-acetic acid is 102. If we multiply this figure by the one just obtained, we have 0.35 g., the weight of aceto-acetic acid in grams from 1 gram of fat.

[2] A small and nearly negligible amount of antiketogenic substance may be derived from glycerol in fat. This is calculated as 0.00057 g. mol. of antiketogenic substance and this is not included in the above ratio.

If the ratio is greater than 2.0, aceton, or aceton and diacetic acid will be found in the urine.

The K/A Ratio Derived from Food Calories.—The physician generally thinks of diet in terms of units of heat energy, known as Calories.[1] Tables which give the Calories corresponding to a given amount of glucid, lipid, or protid foods have been prepared and may be found in different books.[2] Shaffer has calculated the necessary data for the three typical food groups for use in determining the K/A ratio:

1 g. urinary nitrogen = 26.5 Calories.
  Ketogenic value..... 0.010 g. mol.
  Antiketogenic value..... 0.020 "
One Cal. derived from protid =:
  0.00037 g. mol. ketogenic substance.
  0.0075 g. antiketogenic substance.
1 g. fatty acid = 9.509 Calories.
  Ketogenic value..... 0.0036 g. mol.
  Antiketogenic value..... 0.0000 "
One Calorie from fatty-acid = 0.000378 g. mol. ketogenic substance.
1 g. glucid (glucose) = 3.76 Calories.
  Ketogenic value..... 0.0000 g. mol.
  Antiketogenic value..... 0.0056 "
One Calorie from glucose = 0.0000 ketogenic substance
  .001478 g. mol. antiketogenic substance

Then:

$$\frac{K}{A} = \frac{\text{Calories from 100 gs. of fatty acid plus Calories from 100 gs. protid}}{\text{Calories from 100 gs. glucose}} \times \frac{\text{Ketogenic value of one fatty acid or one protid Calorie}}{\text{Antiketogenic value of one glucose Calorie}}$$

Since the right-hand members of the above equation have the values:

$$\frac{0.000377}{.00148} = 0.255,$$

we may give the following equation as the basis of the determination as to whether a subject on a given diet is protected from ketogenesis or not:

$$\frac{K}{A} = \frac{\text{Per cent. Calories from fatty acids + per cent. from protid}}{\text{Per cent. Calories from glucose}^3} \times 0.255.$$

The normal ratio involving no ketosis is $\frac{K}{A} = 1.0$.

[1] Page 596.

[2] Such a table suitable for the pocket is published in the form of a library card, 3 × 5 inches, by Thomas Groom & Co., Inc., 105 State Street, Boston, Mass.

[3] Glucose as starch, sucrose, etc.

The Simplified K/A Ratio of Ladd and Palmer.[1]—This ratio has been derived from the diet protid and glucid. It is as follows:

$$\frac{K}{A} = \frac{\text{Grams of fat in diet}}{0.58 \times \text{gs. protid} + \text{gs. glucid}}$$

A ratio of $\frac{K}{A} = 4.1$ produces ketosis. Roughly, this means that the proportion of fat (K) to glucose (A) in a diet is as 4:1 if ketosis just fails to appear. The figure 0.58 means 58 per cent. of the total food protid is used in the calculation of the ratio. An example of the calculation is:

A male, aged twenty-five years, with evidence of acute diabetes, was treated for five months by dietary methods. As a sample of his diet, March 22d he was given glucids (oranges, turnips, carrots, etc.,) 53 gs.; lipids (cream, ham, and bacon), 247 gs., and protid (eggs, ham, etc.), 50 gs. Substituting in the formula, $\frac{K}{A} = \frac{\text{Gs. fat}}{0.58 \text{ protid} + \text{glucid}}$, we have: $\frac{K}{A} = \frac{247}{0.58(50) + 53} = 3.98$. This is just within the ratio 4.0, which we have stated is safe. On this day the excretion of ketons estimated as aceton was 0.80 g. This is to be compared to ketone excretion of 4.29 gs. when the ratio $\frac{K}{A}$[2] is higher than 5.74.

Summary for the Utilization of Lipids.—(1) Fat, unlike starches and protids is not directly absorbed into the blood, but into the lymphatic system and thence into the blood in the region of the heart. This is probably an adaptation to avoid fat emboli.

(2) Fat differs according to its location in the body: (A) Dépot fat, characterized by having a high content of saturated, neutral fats. (B) Tissue fat, of unsaturated nature and largely phosphorized.

(3) Fat may be formed from starches and sugars, but there is little or no evidence of its formation from nitrogenous substances.

(4) In pathological states, where there is an apparent transformation of protid into fat, it is known that there is either a mobilization of fat already present, or an immigration of fat from outside the tissue.

(5) Cholesterol, included among the fats for physical reasons, is probably a protective substance and performs this function in diseased tissues. It aids in the transportation of fat in the blood by acting with fatty acids to form esters.

(6) Fats are oxidized by beta-oxidation. The last 2 carbon atoms are cleaved from the remainder of the fatty acid to form a

[1] Ladd, W. S., and Palmer, W. W. (Johns Hopkins University and Columbia University), Amer. Jour. Med. Sci., vol. 166, p. 157, 1923.

[2] Ladd and Palmer use the expression $\frac{F}{G}$ (fat to glucose) in place of K and A.

molecule of acetic acid, leaving an acid 2 carbons fewer than before. In the normal animal, the last 4 carbons become oxidized as a butyric acid molecule. In the diabetic this is impossible and these incompletely oxidized butyric acid substances, like aceto-acetic acid and β-hydroxy-butyric acid, accumulate and give rise to acidosis (depleted alkali reserve).

(7) Odd-carbon fats which do not resolve themselves into 4-carbon butyric acid, but rather 3-carbon propionic acid derivatives, are utilized by both normal and pathological animals. These odd-carbon fats do not compensate for acidosis in diabetes.

(8) Ketogenic substances are those which give rise to ketons, aceto-acetic acid, and aceton, which is CO_2-less di-acetic acid. The fats are an example.

(9) Antiketogenic substances are glucids (especially glucose) which are capable of undergoing some change, at present unknown, which permits the 4-carbon chain to be destroyed in the diabetic animal.

(10) In dietetics, it is necessary to know the ability of different foods to produce acid substances and it is possible to calculate the ration of ketogenic to antiketogenic substances. In general, a diet in which not over 10 per cent. of the energy value is from glucids, 10 per cent. from the protids, and 80 per cent. from lipids, will not produce acidosis. A diet in which the amount of fat is not more than four times the amount of available glucose protects against acidosis.

THE UTILIZATION OF THE PROTIDS

Functions of the Protids.—Protid is generally considered as the typical structure-former of the food-stuffs. It must be remembered, however, that other substances not of protid nature form some, at least, of the structure of the cell. Protid makes up about 20 per cent. of the body weight of man. The phospholipids are found in the wall of the animal cell and the characteristic constituents of the cell-nucleus, the chromosomes, are intimately associated with the phospholipids. In the nervous system the phospholipids are characteristic and must play a major rôle. Protids are so intimately associated with glucids that it is difficult in certain instances to say which is the more important. Marsh, Newburg and Hally find that the body requires a minimum of 0.67 g. of protid per kilo of body weight per 24 hrs.

Characteristic Differences between Protids and Other Food Substances. A reserve of glucids in the form of glycogen is found in the liver and muscles and a reserve of lipids in the great dépots of the

omentum and elsewhere; but there is no similar storage of protid. Each tissue absorbs a certain number and amount of amino-acids, but their sojourn is brief. After a meal rich in protids an extra amount called "circulating protid" or "deposit protid" (Lusk) is retained in the body, but it persists only as long as the diet is high in protid. It is true that the protids of the muscle and of other tissues can become utilizable under stress, but this involves a subtraction from the dynamic forces of the body, whereas the withdrawal of fat from the dépots involves at most only a loss of reserve, which in no wise interferes with the activities of the organism. Indeed, the withdrawal of fat may tend to modify the shape of a portion of the body, or change the relative volume, but this is generally compensated for by the introduction of water. The water is doubtless derived from the fat. When fat is oxidized more water is derived from a given amount of fat than is contained in a similar amount by weight. One hundred grams of fat (tripalmitid) gives rise to 109 grams of water when the fat is oxidized. The storage of amino-acids is insignificant compared with the storage of glucids and lipids.

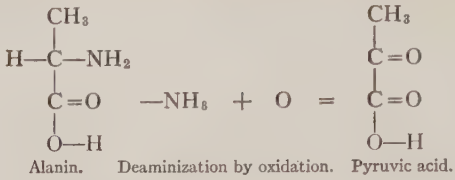
Storage of Amino-acids.—Normally, the content of amino-acids in the blood may be expressed as 18 per cent. of the non-protid nitrogen, that is, about 6 mgs. per 100 mls. of blood. Tissues, especially liver and muscle, are capable of holding free amino-acids. Muscle absorbs these substances from the blood until about 13 times the blood concentration of amino-acids has been reached; that is, muscle absorbs about 80 mgs. of amino-acids per 100 gs. of tissue. The liver holds about twice as much, namely, 150 mgs. per cent. We do not know how these substances are held in the tissues. They are easily removed by water and this leads to the belief that they are not united to the protids or other cellular substances in a chemical manner, or, if so, very loosely. It is probable that the content of amino-acids in a tissue represents a balance between incoming and outgoing amino-acids and not a true storage, as in the case of glycogen and fat.

The Fate of the Amino-acids.—Following the digestion of protid, the amino-acids are passed into the portal system, whence they are carried to the liver. The liver removes relatively large amounts of these acids. Some are converted more or less directly into urea, according to the following scheme:

$$\underset{\text{Glycin.}}{H_2N{-}CH_2{-}COOH} + 3O = \underset{\text{Cyanic acid.}}{H{-}N{=}C{=}O} + 2H_2O + CO_2.$$

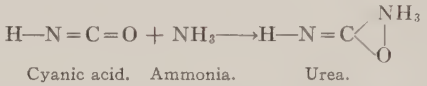
$$H{-}N{=}C{=}O + H_2O = \underset{\text{Urea.}}{H{-}N{=}C(NH_2){=}O} + CO_2.$$

Others are deaminized. This leaves ammonia and a fatty acid, as follows:



Alanin. Deaminization by oxidation. Pyruvic acid.

The ammonia thus formed unites with a molecule of cyanic acid produced from other amino-acids to form urea, thus:

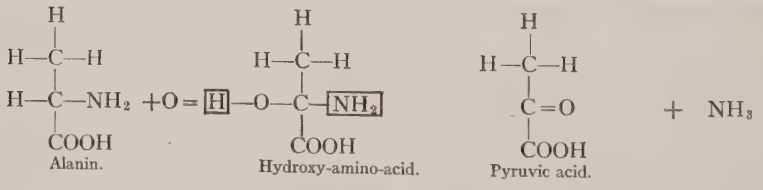


Cyanic acid. Ammonia. Urea.

The Stereo-chemical Relations in Amino-acid Utilization.—Natural amino-acids are characteristically levorotatory, but certain amino-acids, such as *d*-alanin and *d*-aspartic acid are dextrorotatory. If the antipode be given, namely, *l*-alanin, *l*-aspartic acid, or d-leucin, d-phenylalanin, the acid is not utilizable by the body, or only very slowly. If the racemic, or *d-l*-form be given, the body utilizes at first the naturally occurring amino-acid and only later, if at all, the artificial antipode. Generally, the antipode is excreted as such into the urine. Since artificially synthesized amino-acids are generally optically different from the natural ones, or else racemic, that is, inactive, the artificial synthesis of food-stuffs cannot as yet serve mankind as a means of sustenance to any extent.

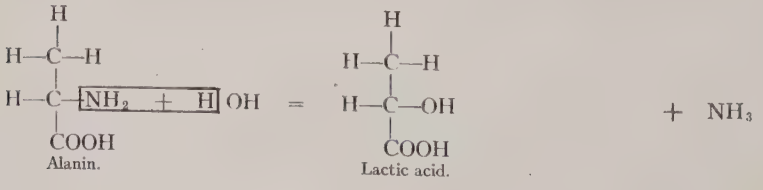
Deaminization—*Deaminization may lead to the formation of:*

1. A keto-acid, like pyruvic acid (deaminization by oxidation):



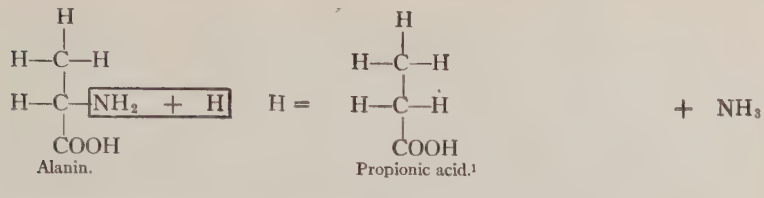
Alanin. Hydroxy-amino-acid. Pyruvic acid.

2. An hydroxy-acid, like lactic acid (deamnization by reduction):



Alanin. Lactic acid.

3. A normal fatty acid, like propionic acid (deamination by reduction):



Probably all of these methods are utilized in the body.

Pyruvic, lactic, or propionic acid may then act as fatty acids, while the ammonia enters into the formation of urea, or of other compounds.

Utilization of Amino-acids in the Tissues.—The amino-acids which escape deamination in the liver pass into the general circulation and are distributed to the various tissues. The tissues which, for some reason, have a depleted supply of a certain amino-acid, characteristic of that tissue, retain their quota of this acid and other tissues remove theirs. Organs which, like the tendons, contain collagen demand a relatively large amount of the amino-acid glycin, but no cystin, whereas the tegumentary organs, like the scalp, bearing hair, utilize a large amount of this sulphur-bearing amino-acid. In this way protid is formed, which, we believe, becomes incorporated into the structure of the cell. The process of absorption of amino-acids from the blood, their introduction into the material structure of the cell, and the breaking down of the structure during which amino-acids are passed to the blood must be one which is occurring continually. The process involves the operation of the laws of mass-action and the physicochemical laws of diffusion and osmosis. These determine the balance between synthesis and catabolism, or the breaking down of the protoplasm. The fate of the amino-acids passed to the blood by the catabolizing tissues is unknown. We do not know whether these acids find their way to the liver or to the kidney,[2] and there become deaminized; or, as, recent evidence[3] indicates, this process of deamination occurs in the tissues. We do not know why amino-acids are lost from the tissues, while others, apparently of the same kind, replace them. The fact that increased metabolism of the muscles does not involve much increase in excretion of nitrogen

[1] Reduction is accomplished as well by adding hydrogen as by withdrawing oxygen.

[2] Benedict's theory concerning ammonia (Chapter XV) demands some deamination in the kidney.

[3] Folin, O., and Berglund, H., Jour. Biol. Chem., vol. 51, p. 395, 1922.

signifies that there is little utilization of the amino-acids during muscle activity. The same may be said of glandular activity. Later[1] we shall discuss the stimulating action of protids on the metabolism of the body, the so-called "specific dynamic action;" this is caused by action of the amino-acids or their direct derivatives upon the cell. We shall also see[2] how glucids exert a sparing action upon the metabolism of the amino-acids illustrated by the action of insulin when the diet is restricted in glucids. The lowered destruction of protid is due to the increased utilization of glucose under the action of insulin.

Utilization of Amino-acids in Abnormal Metabolism.— In diabetes mellitus there is an accumulation of amino-acids in the blood and a corresponding increased excretion into the urine. This is not due to abnormal destruction of tissue, but rather to the failure of the body to make use of the amino-acids. Again, in nephritis, there is an accumulation of amino-acids in the blood; instead of the normal content of about 6 mgs. of amino-nitrogen per 100 mls. of whole blood, a concentration as high as 30 mgs. may develop in interstitial nephritis. This is scarcely due to the fact that the kidney is unable to excrete them normally (urinary amino-acid nitrogen, about 1 g. per twenty-four hours).[3] In hepatic lesions, such as acute yellow atrophy and phosphorus poisoning, the concentration of amino-acids in the urine is abnormally high, owing to the great destruction of tissue in the liver and perhaps also owing to the loss of power of the liver to deaminize amino-acids.

In case of *cancer* in which an increased or unusual method of utilization of amino-acids might be expected, there is none. In the toxemias of pregnancy there is no increase in amino-acid content of the blood unless the kidney is affected, when the effects are those of nephritis occurring at the same time.

We have spoken[4] of the appearance of abnormal amounts of the sulphur-bearing amino-acid, cystin, in *cystinuria*. In addition to cystin, other amino-acids appear and also derivatives of amino-acids like the decarboxylated amino-acid products, putrescin[5] and cadaverin,[6] which impart the peculiar odor to the cystinuric urine. This indicates a more extensive involvement in cystinuria than the amino-acid cystin itself. Alkaptonuria[7] is a striking example of the abnor-

[1] Page 565. [2] Page 566.

[3] Looney, (J. M. Blood changes in acute mercuric chloride poisoning, finds no appreciable increase after total suppression of kidney function lasting ten days. Journ. Biol. Chem., vol. 70, page 513, 1926).

[4] Page 252. [5] Page 260. [6] Page 260. [7] Page 268.

mal disposition of the amino-acids, tyrosin and phenylalanin. These aromatic substances do not follow the usual course of metabolism which involves the utilization of the fatty acid portion and excretion of the aromatic ring, conjugated with sulphuric acid or other conjugant. Instead, the fatty acid portion remains attached to the aromatic nucleus and is excreted in this manner.

Somewhat akin to alkaptonuria is *albinism;* this is the condition in which pigmentation does not occur in the skin, in the hair, nor in the iris of the eye. In alkaptonuria there is the production of a special derivative of the aromatic amino-acids, which, when oxidized, becomes the dark, melanotic substance resembling the oxidized photographic developer, hydrochinon. In the case of albinism the "chromogen," or substance capable of being changed into the pigment of the skin, etc., and derived from the same source as homogentisic acid of the alkaptonuric subject, is present, but the mechanism for its conversion to the pigment by oxidation is lacking. By treating the coat of an albino rat with formalin and alcohol and later by hydrogen peroxid, a brown pigment is produced. In albinism, then, the aromatic amino-acids are not utilized normally.

Increased Utilization of Amino-acids Occurs When They Are Employed for Purposes of Detoxication.—Hippuric acid is increased in the urine if glycin is fed,[1] and if there is sufficient benzoic acid in the body or administered by mouth or by intravenous injection. Substances which otherwise would form urea are used as the source of glycin. Again, arginin, when fed with benzoic acid appears in the urine as di-benzoyl-ornithin, the ornithin being produced from arginin by the loss of a urea radicle;[2] then the ornithin is conjugated with benzoic acid as in the case of hippuric acid. During fasting amino-acids are produced from tissues, and during hemorrhage such production takes place constantly. Concerning the utilization of these amino-acids, we know little, but in studies of lower animals it has been found that they are converted into other tissues, as in the famous case of the Rhine salmon studied by Miescher[3] and in the American counterpart by Greene.[4]

[1] Glycin conjugates with benzoic acid to form hippuric acid. See Lewis, H. B. (Michigan), articles in the Jour. Biol. Chem., volumes 17 and later. Also Jour. Biol. Chem., vol. 18, p. 225, 1924. A photograph of Professor Lewis is given on page 253. See also editorial in Jour. Amer. Med. Assoc., vol. 84, p. 1185, 1925.

[2] Page 259.

[3] Miescher, F., Professor at Bonn-am-Rhein, Germany, one of the founders of biological chemistry.

[4] Greene, C. W. (Professor of Physiology, University of Missouri), who worked upon the Pacific coast salmon.

The Fate of Body Protids.—After the synthesis of protids from the amino-acids, obtained from the foods, or from disintegration of other tissues, sooner or later they undergo hydrolysis and become excreted. Some are destroyed more quickly than others. Thus in order of rapidity of destruction we have: Gelatin, casein, serum albumin, fibrin, globulin, hemoglobin, egg-albumen. Cystin seems to be metabolized more rapidly than other amino-acids, as judged by the ratio $\frac{\text{cystin sulphur}}{\text{urinary nitrogen}}$. In autolysis the globulins are utilized first and then the albumin and other digestible protids. Egg-white affords an interesting example of a lag in metabolism. Thus, the nitrogen from egg-white in an experiment with man was not completely eliminated after six days, whereas that from veal was recovered within half that time.

Amino-acids and Growth.—Certain amino-acids, like lysin, are essential for growth. Others are believed, on good evidence, to be necessary. Cystin, tryptophan, histidin, arginin, and prolin[1] are thought to be indispensable to growth. Definite data regarding the other amino-acids is lacking, but the absence of certain ones, such as lysin, exerts a profound effect in inhibiting growth, which is at once relieved when the missing amino-acid is given as food. Glycin apparently can be made from other amino-acids.[2] In the case of glycin used in making hippuric acid, such glycin is not synthesized from other amino-acids when they are fed, unless it be phenylalanin; there is no increased elimination of hippuric acid when other amino-acids are fed. During growth glycin can be dispensed with and left out of account in feeding.

The inability of certain foods to maintain growth has to do in part with their deficiency in indispensable amino-acids and in part with the absence of vitamins to be discussed later.[3] Gelatin will not afford sustenance beyond a certain time, after which the animal dies. This is because tyrosin and tryptophan are absent, and cystin is present in too small amounts. An exclusive diet of corn protid, zeïn, is insufficient and reference to the table on page 310 shows that this protid is deficient in cystin, tryptophan, and lysin. We shall return to this problem in the next Chapter.

[1] The question of the indispensability of prolin in growth which was proposed by Abderhalden seems answered by the findings of Barnett Sure (University of Arkansas, Fayetteville, Ark.), that prolin is necessary for growth. See Jour. Biol. Chem., vol. 59, p. xvi (Proc.), 1924.

[2] Glycin is synthesized on a glucid diet for detoxications. See page 243.

[3] Page 551.

The Fate of the Nuclear Components.— The "chromatin" of the histologist is identified by the biochemist as the nucleoprotid of the cell. This conjugated protid we have described as a salt in which the base is a protid belonging to the simple protids, protamin and histon; the acid, nucleic acid. In the metabolism of the cell the nucleoprotid is hydrolyzed and the nucleïc acid freed. A cell protid-cleaving enzyme, "autolytic" enzyme, is largely responsible for this action. Next, the nucleïc acid is disrupted by nucleïnases which hydrolyze the tetranucleotid[1] into the four mononucleotids, another group of these enzymes being the nucleotidases. Finally, nucleosidases hydrolyze the nucleosides into the glucid portion (hexose), the purins and pyrimidins. The amino-purins of the cell are oxidized by specific enzymes; one, an adenase, oxidizing adenin to hypoxanthin; the other, xanthin oxidase occurring only in the liver, oxidizing hypoxanthin to xanthin and xanthin to uric acid, while guanase oxidizes guanin to xanthin.

In normal blood all of the purins save guanin[2] have been detected. In the urine, tissue purin, adenin and its products, hypoxanthin and xanthin are represented by uric acid, but the food purins, the methyl-purins from tea, coffee, and chocolate are present as such. Seventy-five per cent. of the total purins of the urine examined by two German workers, Krueger and Salomen, consisted of food purins; the remaining purins were adenin (3.5 per cent.), hypoxanthin (8.5 per cent.), and xanthin (10.1 per cent.). Later work has shown that food purins make up even a larger proportion of the urine purins. Of the urinary uric acid, present in the form of monosodium urate, about one-half is endogenous and the remainder is exogenous, derived from the foods. When glandular substances like pancreas are fed, in which the proportion of nuclei compared with other tissues is high, an increase in uric acid excretion results. Purin metabolism is a function of tissues in general, but of the liver especially. Disintegration of the ingested nucleoprotid takes place in the organs and it is not known whether the purins thus freed appear as uric acid in the urine or whether urinary uric acid is the result of the replacement of tissue purins by food purins which crowd out, as it were, the tissue purins. From the work of Mendel[3] and his associates, it is evident that the course of metabolism of ingested nucleoprotid and that of the nuclei of the cells is identical. Folin has shown that tissues vary remark-

[1] Page 327.

[2] Due to the ever-present guanase oxidizing purin to xanthin.

[3] Mendel, L. B. See photograph, page 408.

ably in their power of absorbing uric acid. The kidney absorbs a greater amount than any other organ.

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CHAPTER XII

NUTRITION FROM THE CHEMICAL STANDPOINT

"The science of nutrition gives promise of making possible the realization of the optimal condition of physical well-being, with all that this implies for the mental achievement, freedom from physical defects, and immunity to many of the ills which result from invasion of the tissues through breeches in the barriers of bodily defense."—*McCollum.*[1]

Nutrition a Primary Function.—Each living thing, plant, or animal exhibits two fundamental functions, self-preservation and per-

Fig. 165.—E. V. McCollum, Professor of Chemistry, School of Public Health, Johns Hopkins University, Baltimore, Maryland. Contributor to the chemistry of substances like phosphorus in the organism and promoter of extensive and pioneer investigations of foods, with especial reference to vitamins.

petuation of the race. Nutrition is essential to the former. The same general method of nutrition which is found in the bacterium is encountered in man, and the differences in food have only to do with a

[1] McCollum, E. V. (Fig. 165), The Newer Knowledge of Nutrition, New York, The Macmillan Co., 3rd ed., 1925.

differentiation in structure, due to environmental restrictions. The bacterium is conspicuously supplied with lipids; the tree is overwhelmingly composed of glucids, like cellulose. The complex organism of man requires a wide variety of nutritive substances of non-nitrogenous and of nitrogenous nature. Biologically, man is not synthetic, for he gains much of his food from organisms, either plants or animals, which have already synthesized the foods, the principal effect being partial oxidation. Man makes use of no foods for energy purposes which do not contain some oxygen. Further oxidation takes place within the body, but the foods are not completely oxidized before excretion, as will be pointed out in Chapter XV. Carbon dioxid and urea are two conspicuous examples of this fact, for both have bonds which are unsaturated, as shown by the formula O=C=O, for carbon dioxid and $H-N=C\begin{matrix}NH_2\\|\\O\end{matrix}$ for urea. Moreover, some have replaceable atoms, like hydrogen in urea, which burns to CO_2, H_2O, and oxides of nitrogen. The foods which are utilized for the production of energy[1] stand intermediate in degree of oxidation, between substances having no oxygen and those which have been completely oxidized. It is not known why the body only utilizes partly oxidized foods or why it does not complete oxidation, thus deriving all the energy possible from the burning.

General Considerations.—Nutrition may be viewed from one of two aspects: (1) From that of the chemical materials composing the body, which replace the structures suffering wear and tear; and (2) from the standpoint of energy of which in man there are two principal types: The energy of heat and mechanical energy. It has already been noted that fats are chiefly concerned with the production of heat and with the production of mechanical energy.

Growth and Maintenance.—Man has a well-defined limit of stature, which is reached by the process of growth. This implies the acquisition of body substance at the expense of the foods. Hence, for the purpose of growth, food is necessary. Furthermore, in order to maintain the body within the limits of standard size, height, weight, etc., food must be consumed; this is known as maintenance. Substances which are indispensable in bringing the body to the stature of the adult are not always indispensable to maintenance of this stature, once attained, or for maintenance of the young when growth is temporarily suspended. Thus, wheat protid, gliadin,[2] serves for

[1] By this is meant not substances like NaCl or H_2O, which do not contribute to the energy of the body.

[2] Page 315.

maintenance, but the percentage of lysin is too low compared with the total content of amino-acids to produce growth. Caseïn, which permits both maintenance and growth,[1] contains 7.68 gs. of lysin per 100 gs. of caseïn, whereas gliadin contains only 0.63 g. per cent. The corn[2] (maize) protid, zeïn, permits neither growth nor maintenance, there being no lysin and no tryptophan and a mere trace of cystin, if any.

The qualitative nature of the chemical relations are more important than the quantitative, as is demonstrated by feeding combinations of amino-acids. Thus, if the two missing amino-acids, tryptophan and lysin, are fed along with zeïn, growth occurs, whereas,

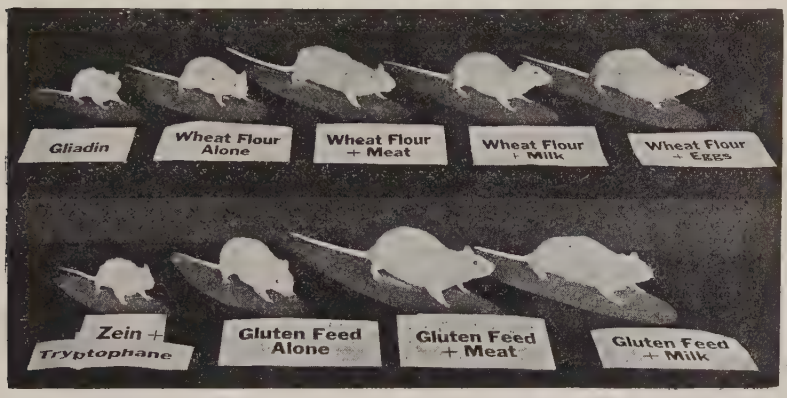


Fig. 166.—Effect of feeding improper and proper combinations of food. (After Mendel, Nutrition, Yale Univ. Press.)

if zeïn is supplemented with tryptophan alone, maintenance but no growth is obtained. Maintenance may be continued for a long time with cessation of growth, when zeïn and tryptophan are fed, but on restoring the full complement of amino-acids by adding lysin, growth is at once resumed. Growth may occur to a certain extent on a purified diet of protid which has a fairly complete suite of amino-acids, but growth is accelerated remarkably when the full complement of amino-acids in the proper quantitative proportions is given. Thus, the albumin of milk, lactalbumin, will cause growth, but if lactalbumin be supplemented by the imperfect protid, zeïn, growth is greatly accelerated.[3] Turning to inorganic substances, a diet defi-

[1] For a period of time; after a certain time special accessories, the vitamins, must be provided, along with salts, etc.

[2] All cereals are called "corn" in certain countries.

[3] For complete description of this work see Mendel, L. B., Jour. Amer. Med. Assoc., vol. 64, p. 1539, 1915.

cient in minerals does not permit growth or maintenance; for unless the diet contain sufficient inorganic substance of a given kind, sooner or later there is a cessation of growth and, still later, a failure of maintenance. The qualitative necessity for definite mineral substances has been demonstrated by attempts to maintain vigor or growth or alkali earth metals, like magnesium and calcium, one being supplemented for the other. The experiments proved that calcium could not be replaced by magnesium.

Purified Diets.—Since the chemical composition of foods is quite definitely known and methods have been devised by which the fundamental units making up the various foods, the amino-acids, etc., may be synthesized from the elements, and since we know, by analysis, the mineral composition of the foods, it would seem possible to mix together glucose, glycerol, amino-acids, and inorganic substances in such proportions that maintenance and even growth would be secured. It has been found by such experimentation that maintenance may thereby be obtained for a time. If the amino-acids are obtained by acid hydrolysis, however, the food is less efficient, owing to the optical properties of the acid hydrolysis products,[1] than when they are obtained by the natural hydrolysis of products under the agency of enzymes, such as pepsin, followed by trypsin. The nutritive value of such artificial foods is limited, and the real value of the experiments in nutrition lies in the discovery of a hitherto unrealized group of food substances which are absolutely essential to maintenance and growth—the vitamins. It was while experimenting on purified foods that Hopkins[2] realized that some factor other than the purified organic and mineral constituents which he had been using was indispensable for both maintenance and growth.

Effects of the Infinitely Small in Nutrition.—Throughout the field of nutrition profound effects are exerted by extremely minute entities. A meager amount of enzyme accomplishes results of great magnitude. An infinitely small amount of certain inorganic substances, like manganese, iron, or iodin, will result in changes in metabolism wholly out of proportion to these amounts. The addition of a minute amount of amino-acid like lysin to a diet deficient in this substance will start the maintenance or growth curve toward a different level. Similar effects of small amounts of material are found throughout pharmacology and therapeutics. Thus, an animal sensitized to egg-white may be killed by subsequent introduction of a

[1] Page 540.

[2] Hopkins, F. G., page 31.

minute amount of the egg-white substance. Ricin, obtained from the same source as ricin-oleïc acid (castor oil), will cause the death of an experimental animal sensitive to it, even though the amount administered is so minute that it cannot be weighed on a delicate balance. Minute quantities of vitamins produce great effects. These are termed by Mendel the "Little Things in Nutrition."

VITAMINS

Definition.—Vitamins[1] may be defined as substances of unknown chemical composition exerting a normalizing influence upon nutrition, maintenance, and growth. Like matter, in general, they are known only by their effects, or by the defects of diets in which they are insufficient or absent. They are probably not synthesized in the animal, but only in the plant. There are several categories and our knowledge of them is as yet very incomplete. Almost every year sees an addition to the list, or the subdivision of a given vitamin, previously considered as a single entity, into two or more vitamins, each exerting a specific function. The author believes that all the specialization of these minute substances, like vitamins, enzymes, antibodies, etc., will be resolved ultimately into simple phases of some single phenomenon; it is inconceivable that a minute cell should possess such profound differentiations as is necessary if we are to assume that it contains a suite of enzymes, many vitamins, a long list of minute determiners of heredity and of sex, and many other things. The chemist is unable to offer any physical basis for such complexity, nor is the histologist able to present evidence of such detail. As knowledge advances, this phantasmagoria of complexity undoubtedly will be explained on simple terms.

At the present time the following vitamins are recognized:

Vitamin A, known also as Fat-soluble A; Antixerophthalmic Vitamin; Antirachitic Vitamin; Growth-promoting, Fat-soluble Vitamin. The chief features concerning this vitamin are:

(1) *Its association* (*but not identity*) *with lipids*, chiefly those of the animal kingdom: "tissue" fats as distinguished from the dépot fats.

[1] The term "vitamin" was originally spelled "vitamine" by Casmir Funk (Polish chemist, formerly in the United States, now in Warsau, Poland), but since the term conveys the impression that these substances are amines, which they are known not to be, it has been suggested that the terminal "e" be dropped, which robs the word of any special significance. Since the term is derived from the Latin *vita* = life, which has a long "i," the word vitamin is pronounced "vyt'a-meen" in the United States. England is prone to follow the Continental method of pronouncing "i" by sounding it "ee," making, therefore, the term "veet'a-meen." In the present book the final e of amine is dropped, since it is from Ammon (the Egyptian Jupiter).

Examples: Fish oils, like shark-oil, dogfish-oil, and oils of other members of the selachians; cod, hake and other members of the teleost fishes; tissues of glandular nature and those in which metabolism is active, like liver, kidney, etc. Plants, leaves, and other parts containing chlorophyl also have this vitamin.[1]

(2) *In its absence, two well-defined phenomena arise: Cessation of growth and cornification of secreting epithelium, notably xerophthalmia.*[2]

(*a*) Although growth ceases, maintenance may be continued, and birds living on a diet deficient in this vitamin will lay eggs from which apparently normal offspring develop. Mammals, however, exhibit lesions in the absence of vitamin A. On the other hand, if minute quantities of some substance containing the vitamin are included in the diet, growth is resumed. Thus, the addition of one drop of melted butter to a deficiency diet for the albino rat will cause the growth curve to pass upward. The reproductive function requires adequate content of vitamin A. Length of life likewise is prolonged on adequate vitamin A diets in experimental animals.

(*b*) Xerophthalmia,[3] an eye condition involving at first lachrymation,[4] photophobia, endophthalmia, then infection and encrustation; finally, depilation of the region around the lids and exfoliation of the epithelium. If the condition is not treated by the addition of an adequate amount of vitamin A to the diet, the cornea will become opaque and the sight of the eye be permanently injured. The conditions just described are exactly those which arose in Scandinavian countries during the Great War, when the inhabitants shipped their butter and cream abroad, leaving skimmed milk for their children. Keratomalacia[5] is found in both children and adults in all countries; this disease resembles the corneal condition of experimental xerophthalmia, due to deficiency of vitamin A.[6]

(*c*) Less manifest disturbances develop in its absence. Calculi of phosphate substance appear in the urinary tract of animals fed a deficiency diet;[7] this condition has not, however, been correlated

[1] See Appendix, Tables of Food Vitamins.

[2] See Walbach, S. B. and Howe, P. R. Journ. Exp. Med., 42:753, 1925.

[3] Greek *xersos*, dry land, and *ophthalmos*, eye; that is, encrustation about the eye.

[4] "Watering of the eyes." Photophobia, dread of light; endophthalmia, receding of the eyeball into the socket; depilation, loss of hair; exfoliation, development of "rawness."

[5] Greek *keras*, horn, and *malakia*, softness.

[6] It is possible that other factors besides vitamin A are important in xerophthalmia; thus McCollum, et al (J. Biol. Chem., vol. 64, page 161, 1925) find experimentally that excessive salt content of foods favors ophthalmia.

[7] Osborne, T. B., and Mendel, L. B., Jour. Amer. Med. Assoc., vol. 69, p. 32, 1917.

with deficiency in vitamin A in man. The relation of fats, calcium and phosphate metabolism, according to which calcium-phosphorus retention in animals is modified by oils like cod-liver oil,[1] known to contain vitamin A, leads us to attribute the formation of calculi to inadequate vitamin A content of foods.

Fig. 167.—Xerophthalmia in a child due to malnutrition during war. It illustrates a baby from the practice of Dr. Bloch, which had suffered from an attack of ophthalmia of dietary origin, and was cured by administration of fat-soluble A, as butter and cod-liver oil. The disease had, however, progressed so far that the sight of the left eye was destroyed. The right eye was somewhat damaged (Bloch[2]).

The possibility of vitamin A deficiency being an etiological factor in the development of rachitis,[3] or rickets, has been quite definitely disproved by modern research. Another factor must be sought and has undoubtedly been found in the effects of radiant energy of the sun or of the mercury-vapor lamp. This will be discussed later on page 559.

[1] Recently it has been shown that oils which do not contain vitamin A, like olive oil, cause Ca fixation.

[2] Copenhagen, Denmark.

[3] Rickets may appear in children or adults, but is far more common in children. In Jefferson Hospital an average of 1 case of adult rickets appears per annum. The disease is manifested in the young by irregular arrangement of the cells normally concerned with the ossification of the epiphyses of the bones, hyperemia (abundant blood-supply), and delayed calcification. Other changes, like altered Ca-P ratio in the blood, interference with intestinal absorption blood conditions, etc., occur.

Chemical Nature of Vitamin A.—We have no certain knowledge regarding this. The chief physical and chemical properties of vitamin A are as follows:

(1) It is heat-stable in the absence of free oxygen.

(2) It is readily oxidized, thereby destroyed and may likewise be destroyed by saturating the unsaturated bonds by bromation or hydrogenation.

(3) Substances containing it may be dried at low temperatures (40° C.) without destroying it to any great extent.

(4) Fats containing it, by boiling with 20 per cent. KOH, may be saponified and the vitamin be recovered from the non-saponifiable fraction. It may be distilled in an oxygen-free atmosphere.

In *dietaries* vitamin A may be supplied by feeding the substances mentioned on page 946. The table shows that many preserved foods contain it in adequate quantities. Practically all canned milks, whether evaporated, condensed, or dried into a powder provide adequate amounts of the vitamin. This is probably the reason why there is so little xerophthalmia or inhibition of growth in the United States traceable to vitamin A deficiency. Koessler, Maurer and Loughlin[1] have described experiments which they interpret as an experimental production of pernicious anemia in rats by restricting them to a diet deficient in vitamin A. Their diet was poor in vitamin A. It was likewise poor in E, and also very deficient in iron, which they do not mention. The butter fat that they employed as a source of A also contains a small amount of E, and the cold liver oil used in other experiments was probably a fairly good source of E, since we have found certain samples of this oil to induce fertility under conditions which are a test for the "fertility" vitamin. Instead of attributing the anemia which they observed to lack of vitamin A, McCollum, Simmons et al. attribute it to deprivation of vitamins A and E_x and of iron.

Test for Vitamin A (Rosenheim and Drummond[2]).—To one drop of a potent cod-liver oil add 1.0 ml. arsenic trichlorid, $AsCl_3$, and immediately agitate the tube. A blue, followed by a purple color ensues if the oil is active. Cholesterol activated by light gives a color, but of different nature. It is possible that some relation may exist

[1] Koessler, K. K., Maurer, Siegfried, and Loughlin, Rosemary. The Relation of Anemia Primary and Secondary, to Vitamin A Deficiency, J. A. M. A., vol. 87, page 476, 1926.

[2] Rosenheim, O., and Drummond, J. C., Biochem. Journ., vol. 19, page 753, 1925.

between vitamins A and D, as indicated by the fact that both give reactions with the arsenic compound (see page 558).

Vitamin B, known also as water-soluble B; Antineuritic Vitamin; Growth-promoting, Water-soluble Vitamin. This vitamin plays a most important rôle in human economy and has figured largely in dietaries all over the world. Of the various vitamins in the food materials used by man, it is the most widely distributed. Its *deficiency* in the diet of the young *produces certain definite effects:*

(1) *Initial Loss of Appetite.*—This is due to some unknown factor, but simple absence of stimulation of the secretions of the gastric juice or of other direct factor is not the cause. As soon as the vitamin is fed a general improvement in metabolism, including appetite and digestion,[1] results. A quantitative relation between vitamin B and appetite holds, 30 gs. of yeast being necessary for the dog to retain normal appetite.

(2) *Failure of Continued Growth.*—Insufficient amounts of vitamin B cause suspension in increase of size, but in other respects development and differentiation continue. If an adequate amount of vitamin B is restored later, the normal size of the organs and of the body as a whole is attained.

(3) *Nervous lesions* (polyneuritis in animals; beri-beri in man) in both young and adult. In experimental animals, like the pigeon, dog, albino rat, etc., lack of vitamin B affects the adult after a varying period of time (two months in the dog) by a paralysis of the extensor muscles of the hind legs and later of the front legs. In the pigeon, muscles of the legs and wings are affected and also the cervical muscles, so that the bird loses control of the motions of its head. These effects are similar to the clonic[2] contractions induced by injection or feeding drugs of the strychnin group, especially when the animal is disturbed. Later[3] we shall discuss the human disease, beri-beri, and compare its symptoms with those of the experimentally induced polyneuritis.

(4) *Lowered Reproductive Function.*—Originally, the absence of vitamin B was correlated with lowered reproductive function, especially of the female, but more careful studies[4] have attributed this effect to another factor discussed on page 560.

[1] Cowgill, G. R., et al. (Yale University), Jour. Biol. Chem., vol. 59, p. xi (Proceedings), 1924.

[2] Greek *klonos*, turmoil.

[3] Page 636.

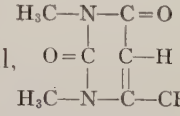
[4] Mattill, H. A., and Congdon, C. C. (Rochester School of Medicine, Rochester, N. Y.), Jour. Biol. Chem., vol. 59, p. xii (Proceedings), 1924.

The *physical and chemical nature of vitamin B* is better understood than that of A.

Fig. 168.—Characteristic attitude of pigeon with polyneuritis (avian beriberi) after three weeks' feeding with a diet of "polished" rice. The pigeon was used year after year as a class demonstration and the extreme effect passed off within a few hours after feeding vitamin B containing foods. The picture represented in Fig. 169 was taken three hours after Fig. 168, and after the pigeon had received a copious feeding of mixed whole cereals, yeast, etc.

Fig. 169.—Pigeon shown in Fig. 168 three hours after feeding a vitamin B rich diet. Three weeks were required to produce the extreme symptoms shown in Fig. 168 and three hours to start the recovery as shown in the above picture. Twelve hours later it would have been impossible to pick this bird from other healthy birds.

(1) Vitamin B is less affected by heat and reagents than is vitamin A. It may be subjected to weak acids at 100° C., without losing its power to counteract deficiency effects. However, alkali, even when quite dilute, affect it profoundly and cause it to lose potency.

(2) Chemically, more is known of its relations than of any other vitamin. Through the investigations of Williams,[1] particularly, following the earlier work of Funk, it has become evident that similarities exist between vitamin B and the pyrimidin, uracil,[2] a component of nucleïc acids. Polyneuritis is improved when β-hydroxy-pyridin, , is administered, a substance which responds to the phenol test of Folin.[3] Likewise, tri-methyl-uracil,  , has been found to offset somewhat the deficiency of vitamin B. However, it must be borne in mind that no single observation concerning the chemical nature of vitamin B has not been challenged. Whatever its nature, it is interesting to note that solutions of dilutions 1:1,000,000 to 2:1,000,000 are potent. Levene[4] has concentrations effective at 15:1,000,000,000.

(3) Distribution of vitamin B among the commoner foods is revealed by reference to page 946. In general, it may be said that in increasing amounts it is found in milk, leafy vegetables such as lettuce, juice of citrus fruits (grape-fruit, orange, etc.), tomato, egg-yolk, glandular organs from animals, and especially in cereals and yeast.

(4) The amount of vitamin B required for protection against any of the above mentioned deficiencies is extremely minute; "it is probably represented by a few parts per million of active body tissues" (Mendel).

Vitamin C, also known as Water-soluble C; Antiscorbutic Vitamin. The importance of this vitamin is recognized both for child and adult:

Effects of Deficiency in Vitamin C.—(1) *Infantile scorbutus* develops when insufficient amounts of the vitamin are supplied, owing to the use of cooked foods and especially of modified milk.[5]

(2) *In the adult* conditions similar to those which occur in the child are found in individuals who live primarily on well-cooked foods or canned products, which do not contain sufficient vitamin C.

[1] Williams, R. R., at present chemist, Roselle, N. J. His chief work was done in the Department of Agriculture, Washington.

[2] Page 330.

[3] The test is non-specific for phenol. See Hawk (page 487).

[4] Levene, P. A. and Van der Hoeven, B. J. C. J. Biol. Chem., vol. 65, p.483, 1925.

[5] Infantile scurvy has been attributed by same authors to absence of certain kinds of light, similarly to rickets, but this is probably not true. See Brit. Med. J., vol. for 1925, 2, page 989.

The disease is known as scurvy and has also been called the "mariner's disease," owing to its prevalence among seamen of past generations. Some food containing this vitamin must be present to maintain normal states.

The *chief physical characteristics of vitamin C* are: (1) It is very susceptible to heat, especially in the presence of oxygen. This is the principal danger of vitamin C deficiency in our modern diet, for practically all processes of food preservation involve high temperatures and oxidation. Some foods, however, are richer in vitamin C than others, and therefore permit considerable loss in its concentration. An excellent example is the tomato; for in practically any form, whether dried, canned in hermetically sealed cans, served partially ripe or very ripe (after being cooked in an autoclave), from 5 to 15 per cent. of the vitamin content is preserved. Potatoes inhibit scurvy, and were it not for the high diet in this form of food among the Irish and Germans[1] of the last century, scurvy would have claimed many lives.

(2) While fairly stable for short periods, it becomes destroyed after a time. This has led to scurvy among members of expeditions who had depended upon preserved foods, even though lime-juice and other food substances were especially provided to ward against scurvy. The latter suffered such loss of vitamin that they became ineffective as antiscorbutic agents.

The *chemical nature* of vitamin C has been studied by N. Bezssnof (Paris), who has isolated a white, crystalline substance from cabbage with which he can both prevent and cure scurvy in experimental animals.

Vitamin D, also called "X" (McCollum), Antirachitic Vitamin. We have stated[2] that to vitamin A was at first attributed the property of obviating rickets, but that recent work has emphasized the necessity of distinguishing vitamin A from another factor, which accompanies it at times and which, for want of a better designation, is called vitamin X (or D).

The symptoms of rickets other than incomplete calcification of the bones, are loss of appetite, loss of body weight, and alimentary disturbances. During recovery, when the vitamin is given, the appetite increases and well-being is manifest to an equal or greater extent than in the stages before the onset of the disease.

[1] This was especially true of the times of the industrial revolutions, depicted by Gerhardt Haupmann.

[2] Page 553.

Characteristics.—Intensive studies on Vitamin D have revealed an intimate relation between the effects of light of definite wave-length (about 2900 Å) and the so-called "Vitamin D." Simultaneously, Steenbock of Wisconsin and Hess (page 646) of New York discovered that unpurified cholesterol obtained from substances known to be antirachitic in the so-called non-saponifiable fraction, itself exerted protective powers against experimental rickets. Moreover, they showed that some substances of non-protective nature became protective if irradiated by direct sunlight or by an arc or mercury vapor quartz lamp giving light of the proper wave-length.

Fig. 170.—Typical rachitic child. Large head. Pot belly. Knock-knees. Flat-foot. (Alfred F. Hess in Abt's Pediatrics, vol. 2.)

Hess has shown that the light alters cholesterol in some way and that "cholesterol oils" which are formed in infinitesimal amounts on irradiating cholesterol, from which it may be separated by precipitation with digitonin are the active principles. Only cholesterol itself and acetyl-cholesterol (acetylation of the secondary alcohol) are capable of being transformed into Vitamin D (Hess). Moreover, as Rosenheim and Webster have shown, only the "protoplasmic" sterols (occurring in the tissues) can be activated, while excretory sterols like koprosterol cannot be activated by ultra-violet light. Rosenhein now has demonstrated that a sterol hitherto known only in ergot (page 269) but now found to accompany cholesterol, is responsible for the antirachitic action.[1] It is probable that the inability of the intestine of a rachitic child to absorb calcium is a potent factor in causing the disease. Further considerations of rickets will be made on page 643.

[1] Rosenheim, O. (Nat. Inst. Medical Research, London). The parent substance of vitamin D. Biochem. Journ., vol. 21 page 389, 1927.

The manner of action of either Vitamin D or light has been explained by Jones[1] as follows: Light or vitamin stimulates the cell to activity. Warburg has shown that active cells metabolize more oxygen and produce, consequently, more CO_2, which increases the acid radicles within the cell. On the exterior of the cell, beyond the cell-membrane, the reaction will become therefore, as Stieglitz has shown on the principle of Donnan equilibrium (page 104), more alkaline; this leads to the deposition of calcium.

Vitamin E, known also as Reproductive Vitamin, Evans' Vitamin, Vitamin E of Barnett Sure.[2] Evans[3] found that by adding natural food-stuffs, like leafy vegetables, etc., to standard diets, the sterility, which developed on the ordinary diets alone was obviated.

Evans and Bishop[1] observed the failure of white rats to give birth to young when fed a diet composed of casein, 18 gs., corn starch, 54 gs., butter fat, 9 gs., salts, 4 gs., lard, 15 gs., and yeast 0.4 to 0.6 gs. daily.

Study of these females revealed the fact that they became pregnant, but resorbed the young. Evans later reported that their experimental diet induced sterility in male rats. He demonstrated the effectiveness of wheat germ oil, among other things, in preventing the death and resorption of the young. It also protects the males against degeneration of the germinal epithelium. Through appropriate experiments, they eliminated the lack of vitamins A, B, C and D as factors influencing the resorption of the young. The substance supplied by wheat germ oil, and certain other foodstuffs which favorably influence reproduction, they designated vitamin X. It is now called vitamin E. McCollum and collaborators[4] have repeated the work of Evans and Bishop, and have confirmed their conclusion

[1] Jones, M. R. (Univ. California), Amer. Journ. Physiol., vol. 79, page 694, 1927.

[2] Wildiers' "Bios" may be identical with this vitamin, but evidence is not convincing. Concerning *Bios*, see Bayliss, cited on page 143. Also see Eddy, W. H., p. 24. Wildiers, E. (Louvain), La Cellule, vol. 18, p. 314, 1901. Funk's vitamin D is probably identical with Wildiers' Bios. See also page 254, note 5, for reference to article by Tanner. Vitamin E must not be confused with Doisy's ovarian hormone (p. 665).

[3] Evans, H. McL. (Professor of Anatomy, University of California, Berkeley), Jour. Metabolic Research, vol. 3, p. 233, 1923. Other references are: Evans, H. M., and Bishop, K. S. J. Metab. Research 1:319 (Feb.) 1922; 1:355 (March) 1922; 3:201 (Feb.) 1923; 3:233 (Feb.) 1923; Existence of Hitherto Unknown Dietary Factor Essential for Reproduction, J. A. M. A., 81:889 (Sept. 15) 1923. Evans, H. M. Proc. Nat. Acad. Sc. 11:373, 1925. Mason, K. E.: J. Exper. Zool., 45:159, 1926. McCollum, E. V., Simmonds, Nina, and Becker, J. E.: J. Biol. Chem., 53:313 (Aug.) 1922. McCollum, Simmonds, Nina, and Becker, J. E.: J. Biol. Chem., 64:161 (May) 1925. McCollum, E. V., Simmonds, Nina, and Becker, J. E.: J. Biol. Chem., 64:176 (May) 1925, chart 2.

[4] J. A. M. A., vol. 88, page 1047, 1927.

that there is a vitamin, a lack of which causes interruption of pregnancy, and produces sterility in males under certain dietary conditions. A detailed histologic study of the degeneration of the testes in rats on their sterility diet has been reported by Mason.

McCollum and collaborators find vitamin E is in some manner associated with iron assimilation. The death of the fetuses in rats on diets deficient in this vitamin is due to a crisis in their iron assimilation, which can be obviated by providing the vitamin E in appropriate amounts from the beginning of pregnancy.

There are two ways in which rats can be caused to recover from this crisis. One is by giving them wheat germ oil and the other is by replacing the ferrous sulphate of the diet by ferric citrate. Ferric salts are far superior as a source of iron to growing rats than are any ferrous salts yet examined.

Although this substance in the leafy vegetables, milk-fat, etc., which counteracts sterility, could not be identified with any of the vitamins, yet the similarity in physiologic manifestations suggested an identity with Wildier's Bios from yeast. Recently Eddy (page 562), has obtained a purified crystalline substance which gives the characteristic effects of Bios. A charcoal adsorption product from yeast has been found by Mattill and Congdon[1] to improve the reproductive function of milkfed female experimental animals; this substance may be Evans' vitamin, referred to above. Finally, Heaton[2] has identified "Bios" with Funk's Vitamin D and has shown that it occurs in similar amounts in brain, liver, kidney, heart and striped muscle; and further that these tissues contain four-fifths of the usual yield of yeast "Bios." Bios occurs in two fractions, (1) not adsorbed to charcoal, and (2) adsorbed to charcoal.

Sure[3] divides the vegetable oils with respect to the presence or absence of a reproductive principle, as follows:

(1) No fertility produced by these substances: Commercial linseed oil, and sesame oil.

(2) Produce fertility, but not lactation: Commercial olive-oil, peach-kernel oil, soy-bean, and peanut oils. Commercial cottonseed oil permits fertility, but only imperfectly affects lactation.

(3) Efficient in producing both fertility and lactation: Wheat-germ oil, hemp-seed oil, yellow corn oil when extracted with ether,

[1] Cited on page 555.
[2] Heaton, T. B. (Oxford, England), Biochem. Jour., vol. 16, p. 800, 1922.
[3] Sure, B. (see p. 560), Jour. Biol. Chem., vol. 63, p. lxxiv (Proc.), 1925.

aceton, or benzene. It is possible that the principle concerned with fertility is different from that having to do with lactation.

Physical and Chemical Characteristics.—As to chemical characteristics of the reproductive vitamin, we have little knowledge other than that previously mentioned. If it is identified as Bios II, then we may give its chemical characteristics as soluble in aceton, not precipitated by baryta-water, but precipitated by lead acetate in neutral or acid solution. Funk[1] has already described the physical and chemical characteristics of the vitamin. It is rather refractory to alkali, may be subjected to autoclaving at 25 pounds pressure for three hours (150° C.); is consumed by yeast, moulds, etc.; this last property differentiates it from vitamin B. Vitamin E seems[2] to be closely associated with protids, but not with any one type. More recently, Eddy[3] and collaborators have isolated a crystalline substance with high melting-point (223° C.), which is not a protid nor protidtemn, is soluble in cold water and in hot alcohol, and causes yeast to grow fifteen to twenty times more quickly than the control.

When white rats are kept on milk food the tendency to sterility can be counteracted by feeding substances containing vitamin E, or by lowering the amount of fat. The size of the ovary and testis is an index as to fertility. Out of:

Forty-one animals fed on high fat ration, 83 per cent. had gonads less than 80 per cent. normal weight.

Sixteen animals fed on low fat ration, 38 per cent. had gonads less than 80 per cent. normal weight.

Ten animals fed high fat + vitamin, 30 per cent. had gonads less than 80 per cent. normal weight.

When the feed contains low amounts of fat, the vitamin must be supplied if fertility is to occur. The vitamin must accompany high fat rations for animals during adolescence and the earlier part of maturity. After sexual maturity, it is not indispensable. The effect of the vitamin, then, is on the primary sex organs and not on fixation of ovum, or concerned with growth.

Avitaminosis.—This term signifies the physiological, or pathological conditions which arise from deficiency or complete absence of vitamins from the food. The importance of this subject is sufficient to warrant special discussion, and this will be undertaken later (Chapter XIV). We may summarize, however, as follows:

[1] Funk, C., and Paton, J. B., Jour. Metab. Res., vol. 1, p. 737, 1922.

[2] Stransky, I. E. (German pediatrist), Jahrb. fuer Kinderheilk., vol. 49, p. 229, 1922.

[3] Eddy, W. H., Kerr, R. W., and Williams, R. R. (Columbia University), Proc. Soc. Biol. and Med., vol. 21, p. 307, 1924.

AVITAMINOSES

Vitamin A:
- Absence: Cessation of growth.
- Deficiency: Atrophic conditions.
  - Anemia.
  - Tendency to infection.
  - Keratomalacia and other eye diseases.
  - Respiratory diseases of upper passages (bronchitis, etc.).

Vitamin B:
- Absence: Beriberi or experimental polyneuritis, after six weeks to four months.
- Deficiency: Loss of appetite (anorexia).
  - Weakness.
  - Anemia.
  - Edema.
  - Subnormal temperatures.
  - Constipation.
  - Colitis.
  - Cardiovascular depression.
  - Malnutrition of the nervous system.

Vitamin C:
- Absence: Scurvy after four months.
- Deficiency: Sallow complexion.
  - Loss of energy.
  - Joint pains ("rheumatism").
  - "Growing pains" of Barlow's disease (infantile scurvy).
  - Mental disorders.

Vitamin D:
- Absence: Rickets.
- Shortage: Rickets, degree varying with deficiency of *D*.

Vitamin E:
- Absence: Lack of lactation. Offspring die.
- Deficiency: Deficient lactation. Offspring lack normal growth.

Vitamins are lost in the following ways:

Vitamin A: By oxidation and by hydrogenation.

Vitamin B: By washing out (boiling in water), by fine milling. Amount of B must be correlated with the starch content of the food.

Vitamin C: Heating, drying, oxidation, twice-heating ("left-overs"), pasteurization of milk, cream, and butter, adding $NaHCO_3$.

Vitamin D: Over irradiation, loss on standing, etc.

Quantity of vitamins necessary for protection:

Vitamin A:
- Dose (for rat, cod-liver oil): Two drops per day adequate to maintain growth.
- Chickens: 1 per cent. cod-liver oil for growth.
- Relative value of certain foods (rat):
  - Beef-fat, four-fifths as good as fresh butter (summer).
  - Mutton-fat, one-fifth as good as fresh butter (summer).
  - Cod-liver oil, 250 times as good as fresh butter (summer).
- Seasonal variation in vitamin content: Cow's milk greatest in summer.

Vitamin B:
- To protect pigeon from polyneuritis:
  - Yeast, 1 gram per twenty-four hours.
  - Egg-yolk, 3 grams per twenty-four hours.
  - Organ fat, like liver, kidney, etc., 3 grams per twenty-four hours.

Peas, fresh, 5 grams per twenty-four hours.
Beef-heart, 5 grams per twenty-four hours.
Brain (beef), 6 grams per twenty-four hours.
Brain (sheep), 12 grams per twenty-four hours.
Beef-steak, 20 grams per twenty-four hours.
Milk (cow), 35 grams per twenty-four hours.

Vitamin C:

To protect against scurvy in:	Guinea-pig, mls.	Monkey, mls.	Man, mls.
Lemon-juice	1.5	1.5	28.0
Cabbage (raw)	1.0	...	0.6
Cabbage (cooked)	5.0	...	10.9
Turnips ("Sweeds")	2.5	...	30.0

Vitamin D:

To protect against rickets:
Dog needs 5 per cent. of food as cod-liver oil.
Rat (white) 3 per cent. of food as cod-liver oil.

THE PROTID FACTOR IN NUTRITION

In the course of twenty-four hours the average normal human being loses, by way of the urine, about 12 gs. of nitrogen. Since about 16 parts of nitrogen are contained in every 100 parts of the average protid substance by weight, about 75 gs. of protid as such[1] are excreted every twenty-four hours. This loss must be restored. Furthermore, a small amount of nitrogen is also lost by the rubbing off of epithelium from the skin, by enzymatic secretions of the intestine, mucus, etc. How much protid must be eaten to restore that which is lost may therefore seem a simple question, since one can analyze the urine, feces, perspiration, etc., and determine the total loss of nitrogen within the twenty-four-hour period. However, there is one factor which complicates this simple method of calculation: Protid, when eaten, stimulates metabolism and causes the destruction of flesh that otherwise would not be affected. This is shown in the following table (Lusk):

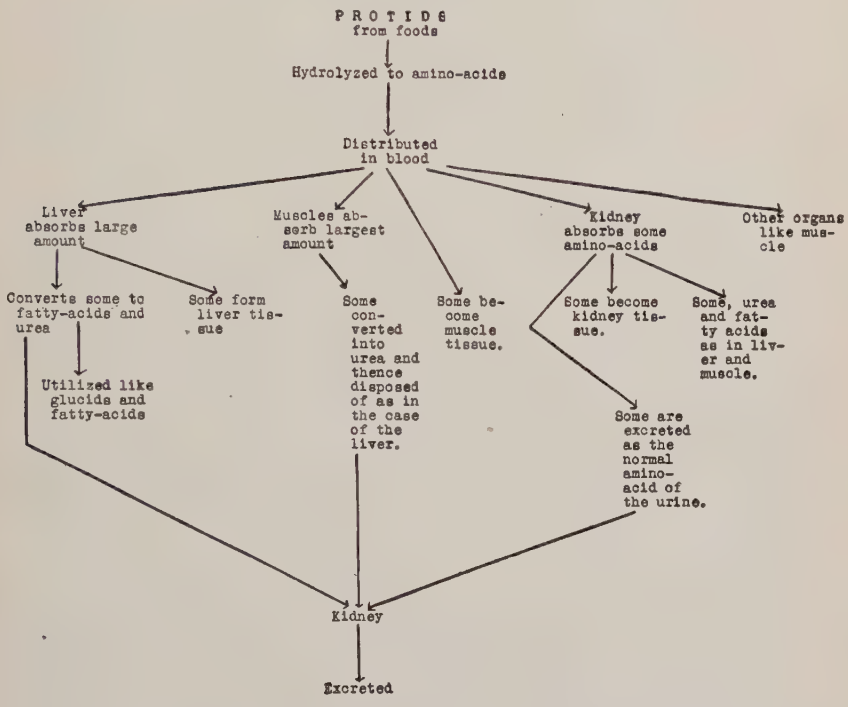
Day.	6	7	8	9	10	11	12	13	14	15
Protid fed	0	0	53	53	53	97	107	119	118	118
As nitrogen	...	...	8.6	8.6	8.6	15.5	17.2	18.3	18.4	18.3
Urinary nitrogen	7.6	9.7	12.9	13.4	14.4	16.8	18.9	20.8	21.5	21.5

In this experiment with man the protid food was given every two hours, the total protid per twenty-four hours being over twice that which is excreted, estimated as nitrogen. In spite of this, *the nitrogen fed never balances that which is lost, for ingested protid causes more*

[1] By this is not meant that protid is to be found in the urine in normal persons, for it is not; the flesh lost during twenty-four hours is 75 gs., but the excretion is in the form of urea, CO_2 and H_2O.

protid to be destroyed. This action is known as the Specific Dynamic Action of protid. It may be considered as increasing the B. M. R. (page 618), 10 per cent.

GRAPHIC SUMMARY


PROTIDS
from foods
Hydrolyzed to amino-acids
Distributed in blood
Liver absorbs large amount
Muscles absorb largest amount
Kidney absorbs some amino-acids
Other organs like muscle
Converts some to fatty-acids and urea
Some form liver tissue
Some converted into urea and thence disposed of as in the case of the liver.
Some become muscle tissue.
Some become kidney tissue.
Some, urea and fatty acids as in liver and muscle.
Utilized like glucids and fatty-acids
Some are excreted as the normal amino-acid of the urine.
Kidney
Excreted

Specific Dynamic Action.—The cause of this stimulating action by protid is held by Lusk[1] to be the amino-acids, especially glycin and alanin; 20 gs. of glycin given to a dog causes a proportionate increase in metabolism. It is not the oxidation of the glycin that produces increased metabolism; it is produced by the action of intermediate substances on the metabolic products of certain amino-acids[2] and not on the acids themselves. The action causes stimulation of metabolism and not oxidation of the amino-acids. Weitzemann[3] believes that specific dynamic action is a true chemical catalysis, which may be studied in the test-tube. He has found[3] that glycin (a substituted ammonium compound) catalyzes the oxidation of

[1] Lusk, G., Medicine, vol. 1, p. 311, 1922.

[2] The dicarboxylic acids, aspartic and glutamic acids; leucin, tyrosin, and probably others do not stimulate metabolism.

[3] Weitzemann, E. J. (University of Minnesota, Rochester), Journ. Amer. Chem. Soc., vol. 49, page 987, 1927.

butyric acid and of glucose (representing, respectively, lipid and glucid) with H_2O_2. In fact a simple protid, like gelatin, exhibits similar properties. It appears that all compounds derivable from glycin have this power. Ammonium hydroxide, contrasted with other hydroxides, such as Na and K and, to a lesser extent, Ca, is capable of catalyzing the oxidation. It is evident, then why glycin appears specific in its ability to exert S. D. A; it is a substituted ammonium compound. The dependence of specific dynamic action on the thyroid has been shown recently by Baumann.[1] Thyroidectomy inhibits the action.

Specific Dynamic Action of Glucids and Lipids.—The glucids and fats under certain conditions cause increased metabolism. The question is whether the cause is similar to that assigned to the protids. The stimulatory effect of glucids and of lipids is realized only when an abundance of these substances is available—a plethora, as Lusk terms it. As in the case of protids, it is not a matter of the oxidation of these substances which produces increased metabolism; for it only occurs when there is an abundance of intermediate products which are not being utilized in the course of work (glucids), or the production of heat (lipids). The nature of the metabolites is unknown. When glucose is converted into fat there is no increase in metabolism. When substances which are not intermediate products of glucose oxidation are given, no increase in metabolism occurs. It is only when there are many intermediate substances on their way to complete oxidation that metabolism is increased. Any factor which removes these substances, such as exercise, causing increased glucid utilization, deposition of glycogen from glucose, etc., inhibits this extra metabolism. Since the height of metabolism is reached about six hours after the ingestion of fat and since this is the time when there is the most blood-fat (Bloor), there is again a plethora.

The specific dynamic action of foods, then, is due to excess of intermediate substances capable of increasing metabolism; in the case of the sugars, it is due to the stored but unused substances; in the fats, it is their abundance which gives rise to extra metabolism; and, finally, in the protids, it is the presence of substances capable of stimulating the cells to undergo metabolism.

The Protid-sparing Action of Glucids.— In diseases of the liver a high diet of glucose-producing substances is indicated and the following discussion explains the principle upon which this is based.

[1] Baumann, E. J., and Hunt, L. (Montefiore Hospital, New York), Jour. Biol. Chem., vol. 64, page 709, 1925.

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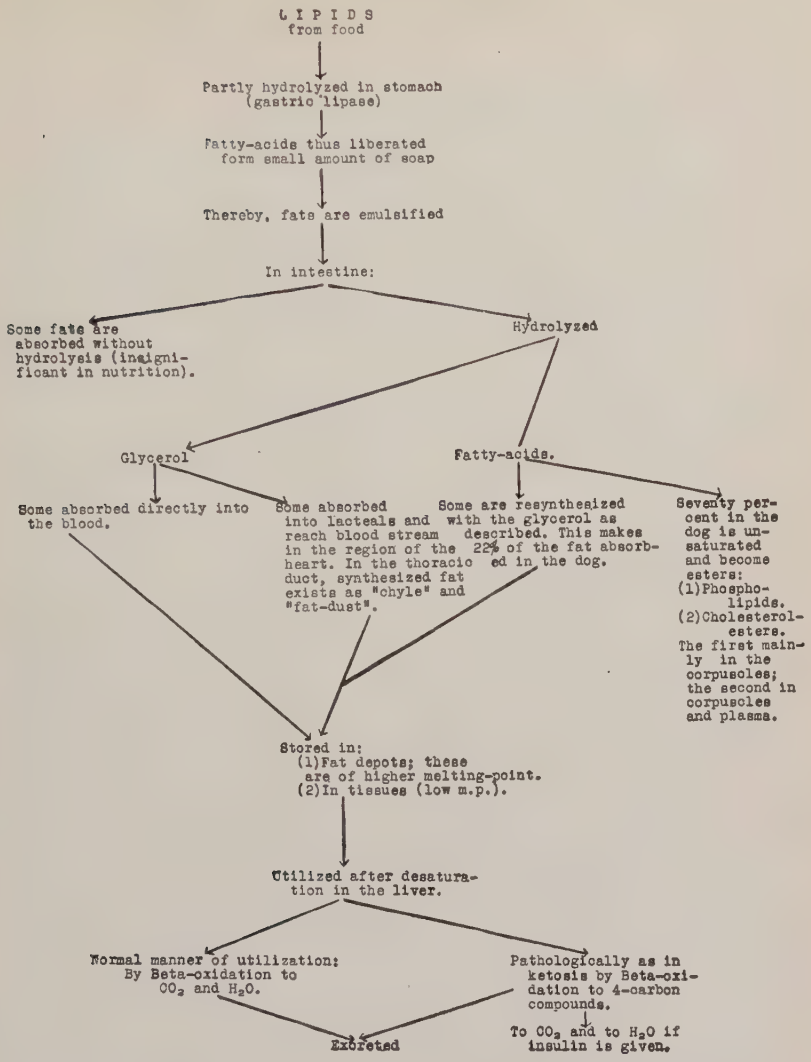

LIPIDS
from food
Partly hydrolyzed in stomach
(gastric lipase)
Fatty-acids thus liberated
form small amount of soap
Thereby, fats are emulsified
In intestine:
Some fats are absorbed without hydrolysis (insignificant in nutrition).
Hydrolyzed
Glycerol
Fatty-acids.
Some absorbed directly into the blood.
Some absorbed into lacteals and reach blood stream in the region of the heart. In the thoracic duct, synthesized fat exists as "chyle" and "fat-dust".
Some are resynthesized with the glycerol as described. This makes 22% of the fat absorbed in the dog.
Seventy per-cent in the dog is unsaturated and become esters:
(1)Phospholipids.
(2)Cholesterol-esters.
The first mainly in the corpuscles; the second in corpuscles and plasma.
Stored in:
(1)Fat depots; these are of higher melting-point.
(2)In tissues (low m.p.).
Utilized after desaturation in the liver.
Normal manner of utilization:
By Beta-oxidation to
CO_2 and H_2O.
Pathologically as in ketosis by Beta-oxidation to 4-carbon compounds.
To CO_2 and to H_2O if insulin is given.
Excreted

We have said[1] that in the case of man the excreted nitrogen can never be compensated for by food protid; that is, man, on a purely protid diet can never be brought into nitrogenous equilibrium.[2] If he could eat enough meat, it would be possible, but this would require the eating of at least 200 gs.[3] of meat in less than one minute. It is possible for a dog to be brought into nitrogenous equilibrium,

[1] Page 564.

[2] Page 564.

[3] About 1 pound of steak as served. A pound is 455 gs., and allowance is made for water, etc.

because he can eat enough meat at one time to compensate for the loss of nitrogen. The following table illustrates this point (Lusk, after Voit):

If a dog eat no meat, he loses 165 gs. body weight.
If a dog eat 500 gs. meat, he loses 99 gs. body weight.
If a dog eat 1000 gs. meat, he loses 79 gs. body weight.
If a dog eat 1500 gs. meat, he loses 0 g. body weight.
If a dog eat 1800 gs. meat, he gains 43 gs. body weight.

A dog can master 1000 gs. of meat in forty-five seconds, but it takes a man twenty-five minutes to accomplish this. If glucids are given with the meat, it is possible to approach an equilibrium, as glucose indirectly replaces the protid destroyed. The following experiment illustrates this:

On the fourteenth day of starvation (man), 7.78 gs. nitrogen excreted.
On the fifteenth day of starvation: cream 300 gs., starch 400 gs. were fed; 7.43 gs. nitrogen excreted.
On the sixteenth day, same diet; 3.58 gs. nitrogen excreted.
On the seventeenth day, same diet; 2.84 gs. nitrogen excreted.

Hence, fat (cream) and glucid (starch) reduced the loss in nitrogen from 7.78 to 2.84 gs. The 5 gs. of nitrogen thus conserved constitutes about one-half the average excretion per day. In the above experiment, fat was fed as well as glucid; this also conserves protid. Certain protids likewise spare protid. Thus gelatin[1] conserves protid to the extent of about 40 per cent. and zeïn 73 per cent. Both of these substances are incomplete protids, lacking certain amino-acids; when the full complement of amino-acids is fed, especially glycin and alanin, the protid-sparing action disappears and the specific dynamic action results. This power of incomplete protids to conserve protid is of great practical importance. It is on this principle that gelatin is prescribed in diabetes and glucids in diseases of the liver.

EFFECT OF DIET ON WEIGHT

Obesity Reduced by High Protid Diet.—Banting[2] found that by restricting the amount of glucids and lipids in the diet, and allowing

[1] Gelatin fed during starvation is retained about a week. The substance retained is not isolated amino-acids, but a complex protid-like compound, but is not a true body protid. See Wilson, H. E. C. (Glasgow), Biochem. Journ., vol. 20, page 76, 1926. Rapport (Rapport, D., Western Reserve Medical School, Cleveland, Journ. Biol. Chem., vol. 70, page 75, 1926) has shown that although gelatin alone has a S. D. A. expressed as +39.4 per cent. (percentage increase over B. M. R., see page 622), gelatin plus its digestion products by trypsin shows S. D. A. of +47.3 per cent.

[2] Banting, William (English lay-experimenter, woodworker by trade, died 1878). Not to be confused with the discoverer of insulin, Dr. F. G. Banting (see page 20).

an abundance of protids, obesity becomes reduced. The explanation of this phenomenon is simple: Protid, when not conserved by the action of glucid or lipid, causes increased utilization of body tissue and, as a result, the fat is burned in larger quantities. Indeed, it is reduced more quickly than the glycogen of the muscles and other portions of the body.

Increase in Body Weight by Diet.—We shall avoid, here, a complete treatment of this topic, for it involves consideration of hereditary tendencies toward leanness, or obesity, and also of the influence of a nervous or phlegmatic temperament. However, generally speaking, flesh may be gained by a diet of protid and glucid, along with a fair proportion of fat, owing to the protid-sparing action of both fat and sugar. Fat is useful in the body for certain purposes which vary with different individuals. It is a sex-limited character in certain races, like the Hottentots. The males select women for marriage according to weight and this selection has resulted in large, fat subjects. Fat is used therapeutically[1] in "floating kidney" to fix the kidney in place. A diet rich in glucids and lipids, with a minimal amount of protid, is prescribed and the patient is kept at rest in bed during the treatment. This favors the deposition of fat. Similarly, in tuberculosis, attention is directed to increase in fat as a safeguard against tissue destruction during the course of the disease. Fat acts as an insulator of heat, in the arctic regions,[2] thus causing its retention; in the tropics it serves to exclude heat; consequently, large amounts of fat in the form of ham, bacon, "side-meat," and sausage are shipped to the warmer countries by the great packers of the United States and South America.

In children, who are fed upon glucid preparations, known commercially as children's foods, there is frequently a mass of subcutaneous and other fat deposited, which, when the rich glucid diet is withdrawn, becomes infiltrated with connective tissue. This may be most undesirable, giving a "pasty" appearance to the skin. Pictures of such children are often displayed in advertisements as unusually healthy children, but they may be quite the opposite. As we have said before, water and fat may replace each other; whenever fat is withdrawn, there is a tendency for the accumulation of water. This helps the body to maintain its form and proportions. In such

[1] Greek *therapeyo*, to serve; hence to administer, or care for.

[2] The popular belief that the chief diet of the arctic is fat is erroneous; it is largely protid, fat serving to protect the protid from destruction. See the accounts given by Stefánsson of the habits of dwellers in the arctic regions in his Life with the Esquimaus and the Friendly Arctic, New York, The Macmillan Company, 1921 and later.

cases the plumpness which is frequently considered desirable gives way to a sallow, haggard condition as the water is gradually withdrawn from the tissues, following the change from a rich diet to a more simple one, or as age advances.

Fat is looked upon more and more as undesirable when it exists in excessive amounts and several diseases of the organs of internal secretion, like the pituitary and genital organs,[1] involve a deposition of fat which is pathological. A fat subject usually has more difficulty at childbirth. Diabetes mellitus usually occurs in fat subjects and, as a rule, the fatter the subject the greater is the probability of acidosis, since the fat may give rise to acids like those of the butyric series.[2]

THE GLUCID FACTOR IN NUTRITION

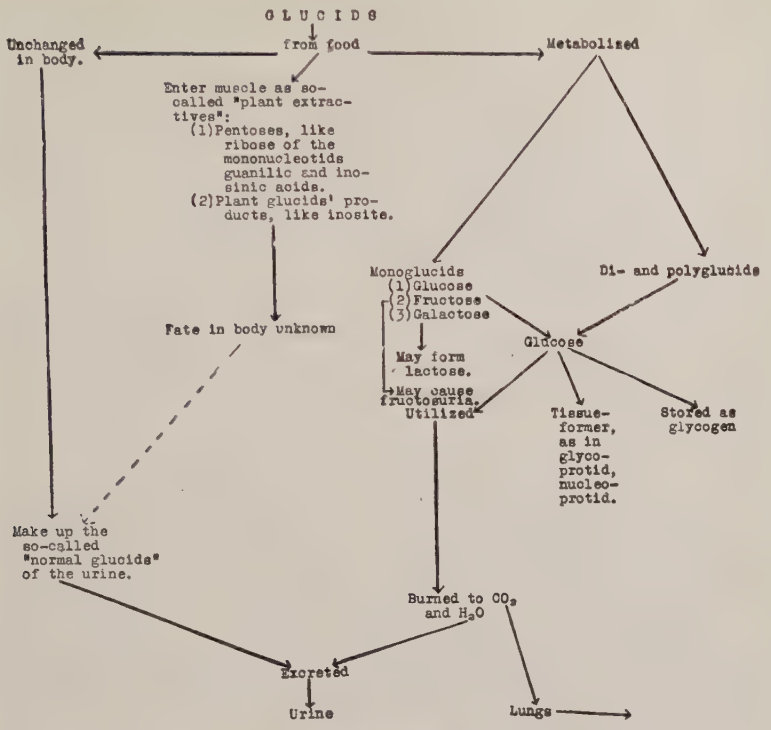
Carnivores live largely on flesh diets, but it is known that they obtain a certain amount of glucid from the glycogen in the muscles and other tissues; from the glucid portion of the glycoprotid, mucin, and from that of the nucleoprotid of the nucleus. It has been supposed that a certain content of glucid in foods is essential to the burning of the fats.[3] However, Osborne and Mendel[4] have shown that rats can furnish from their own tissues, supplemented by glucid-free lipid and protid foods, whatever glucid is necessary for growth and maintenance. It is not necessary, then, that the food contain preformed glucids; these can be obtained from endogenous sources such as protid. Analyses of the whole body of rats fed upon a practically glucid-free diet gave a total glycogen content of 0.09 g. per 100 gs. of body weight as compared to 0.12 g. per 100 gs. per cent. for rats on the usual diet of starches, fats, and protids. It might be supposed that the glycerol of fats, which is practically a glucid, replaces the preformed glucids, but Osborne and Mendel have found that this is not the case.

We have previously discussed the rôle of glucids in:

1. Structure formation, as glycoprotids, nucleïc acid, etc.
2. In the metabolism of fat, where glucid is necessary for lipid metabolism.
3. In muscle contraction, through the agency of lactic acid.
4. In protecting protids from metabolism through "protid-sparing action."
5. In bone formation, by the transfer of phosphorus.

[1] Figs. 208 and 209. [2] Page 80. [3] Page 532.
[4] Osborne, T. B., and Mendel, L. B., Jour. Biol. Chem., vol. 59, p. 13, 1924.

GRAPHIC SUMMARY



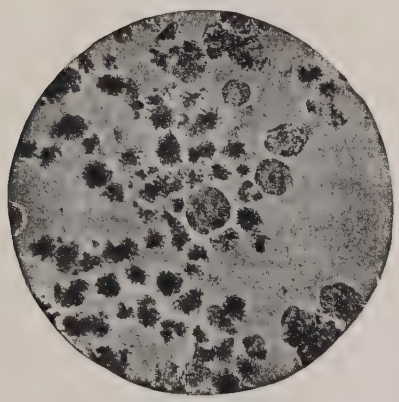


Fig. 171.—Butter, adulterated with 10 per cent. cocoanut oil. Crystals magnified 125 times. (From Woodman, Food Analysis, McGraw-Hill Book Co., New York, N. Y.) Compare Fig. 93.

Glucid and Lipid in Nutrition.—It is not known what relation exists between the burning of fat and the metabolism of glucid. Perhaps neither is oxidized as glucid or lipid, but instead as a common

glucidtemn or lipidtemn. This relation will be made more apparent in the next Chapter, when energy considerations will be discussed, but here it may be said, quoting Lusk: "There seems to be little difference in the efficacy of the body as a machine, whether fat or carbohydrates are used as fuel," and as we shall see it is not necessary to take into consideration the isodynamic value[1] of each substance. On a diet high in fat, however, fatigue appears earlier than when the diet is largely composed of starches, probably, owing to the formation of acids. The K/A ratio[2] is abnormal, tending to the formation of ketogenic compounds. The diabetic, who cannot utilize glucose, fatigues early in muscle work. As a general rule, work done on a glucid diet is 10 per cent. more efficient than on a lipid diet; that is, it is done on an expenditure of 10 per cent. less energy.

INORGANIC SUBSTANCES IN NUTRITION

Modern biochemistry, with its new delicate methods for quantitative analysis of mineral substances, makes it possible to estimate the importance of such substances in nutrition more accurately than was formerly possible. It has been demonstrated that inorganic substances play as important a part in the economy of the human being as do the organic. We shall present in review examples of important mineral substances:

Sodium Chlorid.—Salt-free diets cause death if carried beyond a certain limit; a pigeon dies within a fortnight, while a dog may live nearly twice that long. Wild animals travel many miles to a "salt-lick" to obtain salt. On a normal diet about 15 gs. of NaCl are excreted by the kidney; a small amount is excreted through the skin and a smaller amount through the secretions of the alimentary canal, amounting in all to about 22 gs. We should expect this to represent the required intake of NaCl in the food, but it has been shown that man may live apparently normally on not less than 5 gs. per day or about one-fourth that usually taken. The average salt intake in the United States is less than that in Europe, but intake in NaCl does not vary widely in different countries as does the intake of other minerals.[3] Growth occurs in experimental animals on a low chlorin diet and, in general, it may be said that a sodium chlorid deficit is borne more easily than a deficit of other minerals. Attempts have been made to reduce the salt content of blood in cases of hypertension

[1] Page 603. [2] Page 535.

[3] See the discussion of phosphorus and calcium, page 647.

(increased blood-pressure), or in pyloric spasm or stenosis. Such a study has been made by Bernstein[1] using salt-free diets in hypertension, previously suggested by Ambard,[2] Allen,[3] and others. Blood-plasma chlorid does not vary in a characteristic manner in salt retention, owing to the compensatory functions of the blood which regulate its saline content. The urine is a more faithful index of the metabolism of salt in the body. The following table of salt content as NaCl serves to give the reader an idea of foods which can be used for purposes of reducing the salt of ingested substances:

Foods containing 1: 1000 or less NaCl.	G. per 100 gs. substance.
Apple-sauce	0.0
Beets	0.1
Chicken broth	0.07
Saltless butter	0.0
Carrots	0.10
Coffee	0.06
Fruit	0.01
Ice-cream	0.17
Olive-oil	0.0
Potatoes	0.03
Rice	0.0
Shreaded wheat	0.0
Sugar-cane	0.0
Toast (salt-free bread)	0.0

A typical protocol of salt-free diet treatment for hypertension is given (case of Mrs. Mary T., service of Drs. Hare and McCrae, Jefferson Hospital):

Date.	NaCl in diet.	Urine, NaCl, g.	Blood-plasma, NaCl., mgs. per cent.	Blood-pressure,[1] A. M.
14.10	0	0.764	587	210/110
16.10	0	1.392	...	206/120
18.10	0	0.577	...	190/110
20.10	0	0.388	624	198/120
22.10	0	0.338	...	210/120
24.10	0	0.679	...	170/116
26.10	0	0.308	577	144/100

[1] The first figure is the systolic pressure and the second the diastolic.

[1] Bernstein, M. (Jefferson Hospital, Philadelphia). Therapeutic Gazette, vol. 49, W. S., p 241, 1925. Since Na and K are antagonistic (see page 138) and since K accumulates under many conditions in which Na is decreased, it is possible that the favorable effect of a low chlorid diet may be to cause a *relative* increase in K which may cause lowering of blood pressure.

[2] Chapter XVI.

[3] Allen, F. M. (Physiatric Institute, Morristown, N. J.), Jour. Metabol. Research, vol. 2, p. 429, 1922.

It is obvious that the blood-pressure falls after a saltless diet has been administered for a few days. The age of the subject was fifty-seven, and her expected blood-pressure would be about 160/100, so that 144/100 is well within the normal limits.

In such restrictions of intake of chlorid, the blood chlorid varies but little from the normal (0.50 g. NaCl per 100 mls. whole blood) to 0.36 g. per cent. The effect upon the secretion of gastric HCl is slight. In cases of diabetes mellitus the chlorid content of the blood falls to 0.46 g. per cent. In nephritis, especially in that form involving edema, it increases, owing to the decreased permeability of the kidney, the figure ranging from 0.50 g. per cent. to 0.61 g. per cent. whole blood.

We have spoken of the rôle that chlorids play in the production of alkali reserve[1] and have called attention to the importance of chlorids in balancing the osmotic relations of the various salts in the blood.[2] *The optimal concentration of the salts in the blood compared to the concentration of sodium is practically that of sea-water.* The loss of chlorin, Cl^-, from tissues causes paralysis; chlorin may be removed by giving sodium bromid or calcium chlorid, by administering low NaCl diets followed by diuretics, or by causing loss by exosmosis alone. Sodium chlorid causes relaxation of involuntary muscle. Again, in absorption, substances like glucose and amino-acids pass into the circulation from the intestine more quickly in the presence of NaCl than when alone. In starvation, NaCl is conserved.[3] In nutrition, these demands must be met.

Calcium.—The *minimal daily requirement* for human adults is given by Sherman[4] as 0.45 g., expressed as CaO, with a variation of about 0.06 g. for different subjects. Owing to the loss of calcium in stools; the possible deficiency in vitamin D or parathyroid secretion which regulates the retention of calcium in the body; the lack of adequate magnesium which causes Ca retention; and for other reasons, adequate supply of calcium from food must be insured. This is not always the case. One of the "other reasons" is the protid factor; Sherman finds that at least 1 g. of calcium should be furnished for every 100 gs. of food protid. This is not realized in the ordinary diet in the United States, for he has shown that the average content of human food in CaO on an average diet[5] is only 0.92 g. This should

[1] Page 84. [2] Page 122. [3] Page 589.

[4] Sherman, H. C., Jour. Biol. Chem., vol. 44, p. 21, 1920.

[5] The diet is estimated on a caloric basis of from 2500 to 3000 Cals. See page 593. Such diets are overbalanced with cereals (bread, breakfast foods, etc.) low in Ca.

be constrasted with the diet of Europeans in which milk is used more freely. The Finnish diet contains about four times the amount of CaO that our diet contains. The following table gives the distribution of calcium as CaO in various foods (after Lusk from Sherman and Gettler):

	G. CaO per 100 gs. substance.
Beef	0.0038
Eggs	0.0670
Milk	0.1200
Oatmeal	0.0930
Rice, white	0.0080
Flour, white	0.0260
Flour, whole-wheat	0.0440
Beans, lima	0.7100
Cabbage	0.0490
Peas	0.1000
Potatoes, white	0.0110
Turnips	0.0640

Milk furnishes the largest supply of calcium, and the legumes afford an ample supply. For children a gram of calcium per twenty-four hours is requisite. Of this, about one one-hundredth is retained to build the bones. Food calcium obtained from sources other than milk is less readily utilized than milk calcium and on a diet of legumes and other foods, where the calcium supplied is about half that afforded by milk ingested at the same time, the amount of calcium retained and excreted varies more than when milk is the chief bearer of calcium. This point should be remembered when lime-water, $Ca(OH)_2$ is advocated as an agent that brings in more calcium than milk, for Ca is less utilizable in this form. It is advisable to overshoot the mark in administration of calcium and in order to insure to children an adequate amount of Ca, a quart of milk should be given per twenty-four hours, and also vegetables, for calcium is not utilized unless vitamin D is present and the parathyroids intact. Unless the cow receives a diet of legumes (clover, alfalfa, etc.) and not one largely of grasses, cereals, etc., the milk may be deficient in Ca. If the food contain insufficient Ca, the cow, like man, draws upon its own supply of calcium in the bones.

Although calcium is more available in the form of milk calcium, the statement is not meant to lend support to the view once prevalent that calcium to be of use in the body must be administered in the form of some organic combination. *Inorganic calcium*, as in lime-water, is used, but less readily.

The *relations of calcium to disease is important*, but we shall defer such discussion until a later chapter.[1] Suffice it to say here that calcium enters into the phenomena of:

1. Hardness of the teeth.
2. Deficiency in rickets, osteomalacia, craniotabes, acromegaly, etc.
3. Calcification in the arteries in old age (arteriosclerosis).
4. Nervous disease, Ca acting as depressant.
5. Toxic conditions, like oxalic acid poisoning in which the calcium is depleted by being formed into calcium oxalate.
6. Blood coagulation, milk coagulation, rigor mortis, etc.
7. In tuberculosis, in which there is an increased excretion of Ca.
8. Physiologically, in the regulation of the heart by way of the vagus nerve, calcium being necessary for this action.
9. Parathyroid disease, these organs controlling calcium metabolism.
10. Acid-base equilibrium in the body.

Discussion of *the participation of calcium in metabolism* will be deferred until the end of the present Chapter, because other inorganic substances participate in this important process.

Magnesium is metabolized largely with calcium, in its retention and excretion. When fed, it causes retention. Increased excretion of either magnesium or calcium involves similar increase in the other. However, physiologically, the two stand antagonistically. Magnesium sulphate is an anesthetic[2] and paralyzing agent, the effects of which are counteracted by calcium. Magnesium occurs in the tissues, blood[3] and bones; the erythrocytes and voluntary muscle contain more Mg than Ca. The requirement in twenty-four hours is approximately 0.60 g. expressed as MgO. It is excreted through the kidneys in amounts of 0.2 g. daily. The loss through the kidneys is practically wholly that of the magnesium contained in the metabolized muscle plus a small amount from bone. The magnesium of the blood, like the other salts, serves to maintain the proper osmotic relations. It acts similarly to calcium in the regulation of the heart rate. Increased concentration of magnesium retards coagulation

[1] Page 643.

[2] The well-known studies of Meltzer (S. J. Meltzer, physiologist, Rockefeller Institute, New York, died 1920) and Auer (St. Louis University) showed that in the rabbit, 1.7 g. of $MgSO_4$ for every kilo of body weight causes deep anesthesia and paralysis within half an hour, but the effect can be counteracted within five seconds by injecting intravenously 8 mls. of 3 per cent. $CaCl_2$. See Meltzer's Life in Memorial Number of Proc. Soc. Exp. Biol. Med., vol. for 1921.

[3] Average normal amount, 0.003 g. per 100 mls. of blood plasma.

whether in milk, blood, or rigor mortis. In tetanus or "lock-jaw," caused by Claustridium tetani, magnesium lessens the number of convulsions, probably because it is mobilized under these conditions from the tissues. This action is augmented by giving magnesium sulphate by mouth or intravenously.

Potassium plays an important rôle in antagonizing the sodium and calcium of the body; it exerts a paralyzing action,[1] and in cases of potassium deficit the vagus nerve is incapable of exerting its regulatory effect upon the heart. Whenever there is a large destruction of tissue, as in starvation,[2] the excretion of potassium is increased because this substance is freed from the tissues. On the other hand, the sodium excretion is small, since sodium occurs normally in body fluids. Whenever potassium tends to accumulate, as in high vegetable diets, sodium is eliminated in the urine in increased amounts and potassium (standing above sodium in the electrochemical series) replaces it in the sodium compounds of the blood. Moreover, the ionic mobility[3] of potassium is higher than that of sodium; it is indeed greater than any cation except hydrogen. That is, potassion is transported more quickly than any other cation with the exception of H^+ and this may account for the powerful pharmacological action of potassium. During recovery from wasting diseases, during mountain climbing which is attended by an increase not only in muscle tissue, but conspicuously in erythrocytes, and[4] in other physiologic states in which new tissue is being formed, potassium is retained.

Phosphorus is an extremely important element in the economy of the body. It is characteristic of the cytoplasm, but is absent, except in organic form, from the nucleus. It occurs in the nucleoprotid. For maintenance in the adult, 0.88 g. as phosphorus is the minimum requirement, for an average of 0.78 g. is excreted in the urine and a small amount also in the perspiration and through the feces. We encounter the same condition found for other components, namely, a fairly uniform concentration under varied conditions. A safe daily intake is about 1.5 g. of phosphorus. In the blood[5] the

[1] Diets rich in potassium have been proposed to present convulsions of epilepsy. Such diets are extremely hazardous as in many cases they have proven fatal.

[2] The urinary output of nitrogen is low in starvation until the "premortal rise" simply because there is little protid metabolism, the protid food not entering the body, but there is relatively much tissue destruction during these periods (page 627).

[3] Page 56, note 1.

[4] Schneider, E. C. (U. S. Aviation Service, New York, N. Y.), Physiol. Revs., vol. 1, p. 631, 1921.

[5] Anderson, G. H. (Glasgow), Biochem. Jour., vol. 17, p. 43, 1923.

normal amount for children is 0.005 g. per cent.[1] plasma and slightly less for adults. In bone lesions[2] and in repair processes in general[3] there is an increased mobilization of phosphorus in the blood, signifying the active participation of this element in bone repair and tissue growth. On the other hand, in rickets, there is usually a lower amount of blood phosphorus, although there are exceptions. Foods determine the phosphorus metabolism. Fats and oils, such as butter-fat, cod-liver oil, olive-oil and linseed oil, cause retention.

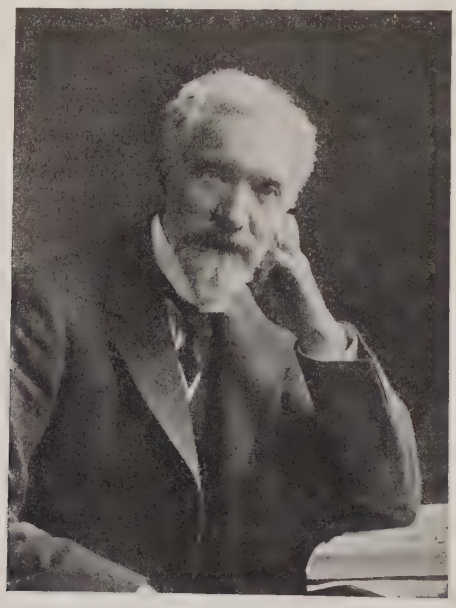


Fig. 172.—A. B. Macallum, Professor of Biochemistry, McGill University, Montreal, Canada. Contributor to mineral metabolism and methods for determination of inorganic substances.

Neither vitamin A nor the associated antirachitic factor is responsible because olive-oil does not contain fat-soluble or antirachitic vitamins. Foods which are rich in phosphorus do not necessarily cause phosphorus retention in the body. The cereals are poor in calcium but rich in phosphorus, yet a diet of cereals does not cause a retention of this element, but rather a failure to retain it.[4] Regard-

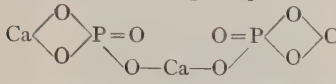
[1] This is too high according to Eddy and Heft, who find 0.0029 g. See note 3, below.

[2] Tisdall, F. F., and Harris, R. I., Jour. Amer. Med. Assoc., vol. 79, p. 884, 1922.

[3] Eddy, W. H., and Heft, H. L. (New York Hospital), Jour. Biol. Chem., vol. 55, p. 12 (Proceedings), 1923.

[4] Husband, A. D., Godden, W., and Richards, M. B. (Aberdeen), Biochem. Jour., vol. 17, p. 707, 1923.

ing the form in which phosphorus is utilized, it is evident from investigations that it need not be organic. Growth occurs on a diet free from organic phosphorus. This means that such phosphorus compounds as lecithin, nucleïc acid, etc., which form during growth must be made from inorganic phosphorus. When demanded, the blood can mobilize phosphorus from the bones. However, the phenomenon of phosphorus metabolism is complex and factors which are unsuspected may control its presence. Thus, in certain pathological conditions of the intestine, especially if fat digestion is interrupted, owing to the absence of bile, as in obstructive jaundice, the normal course of excretion of phosphorus in the form of fecal tricalcium phosphate



is changed; the phosphorus is freed from its association with calcium and is absorbed into the blood from which it is excreted into the urine. In this case 70 per cent. of the feces dry weight consists of fatty acids, or their derivatives, calcium soaps, the calcium having been separated from the phosphorus and combined with the fatty acids to form the soaps, while the phosphorus is left to become absorbed into the blood.[1] The calcium-phosphorus ratio in rickets and other forms of disease will be discussed later.

The *probable relation between phospholipids and the nucleïc acids* has been mentioned; also the participation of phosphorus in the metabolism of the sugars (page 176); and the importance of the phosphorized fats in the formation of the chromatin of the cell (concerned with heredity; see page 328).

The *participation of phosphorus as phosphate* in the maintenance of the *neutrality of the blood*[2] has been mentioned. The reaction of the urine is largely related to the phosphate content. Acid-base relations will be considered later.[3] Concerning phosphorus in the foods, there is no danger of a deficit in the American diet.

Iron.—The primary use of iron in the body is in the composition of hemoglobin. In the fetus iron is supplied by the mother and stored in the liver, bone-marrow, and spleen from which it is withdrawn immediately after birth as long as the infant is on a milk diet.[4] More iron seems to be laid up in the organs of the fetus than is necessary, since the child, after birth, loses more iron than it takes in, the average loss being about 0.25 mg. per day. The chick derives iron

[1] Tefler, S. V. (Glasgow), Biochem. Jour., vol. 15, p. 347, 1921.

[2] Page 82. [3] Page 590.

[4] See the low concentration of iron in milk given in the table above.

from the yolk of the egg which contains almost as much iron as steak. The average amount of iron derived from foods in the United States is about 0.025 g. per day, but in European diets the amount is larger. The following table gives the concentration of iron derived from the common foods:

CONTENT OF IRON IN COMMON FOODS
(Sherman and Gettler, with additions)

	G. per 100 gs. substance.
Steak	0.0038
Eggs	0.0030
Milk	0.0002
Oatmeal	0.0037
Rice	0 0007
Wheat flour	0.0015
Whole-wheat	0.0052
Beans, lima	0.0072
Beans, green	0.0016
Cabbage	0.0009
Peas, dried	0.0056
Potatoes, white	0.0012
Lettuce	0.0021
Spinach	0.0038
Turnip	0.0006
Apples	0.0003
Raisins	0.0036

The *chief sources of iron* in the average diet are beef and muscle meats, eggs, whole cereals, legumes, and leafy vegetables. Whatever leads to the increase in red blood-corpuscles (hemorrhage, high altitudes, etc.) causes a retention of iron. In the blood, iron occurs wholly in the hemoglobin in the corpuscles in an amount of 0.003 g. per 100 mls. blood. It probably is a catalytic agent[1] in oxidation. The dependence of glutathion upon iron has been metioned before. The functions of the so-called "peroxidase" reactions with benzidine, guaiacum, etc., used to detect the presence of blood has been pointed out on page 118. It is for this reason that iron causes improvement in certain anemias, in which the hemoglobin of the blood is below normal. Its administration is only useful therapeutically to supply a deficiency in its content. Recently, favorable results have been reported in the ingestion of fresh blood in pernicious anemia, but it is not known whether the iron content is a factor or not.

Any *excess iron* is stored in the marrow, spleen, kidney, and liver and in other organs concerned with the metabolism of blood. Iron is always utilized, whether it is inorganic or organically bound. In

[1] Page 118.

milk, iron is wholly associated with organic substances.[1] In the animal body, iron is found in hemoglobin, but also in such protid combinations as hematogen[2] in the yolk of eggs and ferratin[3] in the liver, the latter being indigestible. Iron is retained in the body in larger quantities when vitamins are adequate in food, than when they are deficient, but some iron will be retained, even in the absence of these substances.

Iron as a Therapeutic Agent.—After *splenectomy* concentration of iron in the blood is diminished and iron is administered in order to supply the deficit. There is, however, a protective device by which iron is conserved; it must be used over and over again, as much as possible. Elimination of iron from the foods causes a definite lower-

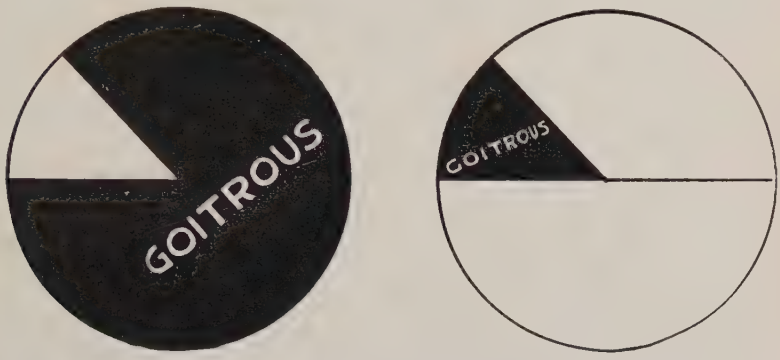


Fig. 173.—Population before and after iodin treatment (after Mendel, Nutrition, Yale University Press).

ing of iron in the body, beyond which, however, further loss does not occur, unless the deprivation of iron occurs over a long period. This minimal level of iron varies in different subjects and under different physiological and pathological conditions. In *pernicious anemia*, in which new erythrocytes fail to form, iron therapy is frequently without avail.[4] *Chlorosis*, a disease of the female about the time of puberty, involves a lowering content of blood iron, and with some cases improvement is found by giving iron, but there is no evidence that chlorosis is produced by iron deficit.[5] The so-called *wet beri-*

[1] Kugelmass, I. N., Bull. Soc. chim. biol., vol. 4, p. 577, 1922.

[2] Hematogen of Bunge (professor at Basle, Switzerland, deceased).

[3] Ferratin by Schmiedeberg (professor at Strassbourg, France).

[4] See, however, Gibson, R. B., and Howard, C. P. (Iowa City, Ia.), Arch. Int. Med., vol. 32, p. 1, 1923.

[5] For a discussion of iron in anemias, see Jour. Amer. Med. Assoc., vol. 81, p. 1022, 1923. It is of interest that for an unknown reason chlorosis is less frequent than formerly.

beri is a disease attributed to foods low in iron. Recovery from *hemorrhage* is apparently affected by the amount of iron in the food. Experimental animals fed upon white bread with its low content of iron recover from hemorrhage more slowly than control animals fed upon green plants. Iron may play a part in the characteristic symptoms of certain diseases. We know that there is a lowering of blood iron in hypothyroidism,[1] a disease in which the oxygen consumption and metabolic rate are likewise lowered.

Excretion of Iron.—Iron is excreted largely in the feces. In the urine iron is small in amount (0.00008 g. per 100 mls. urine).

Iodin.—The importance of iodin in the diet is emphasized by the high incidence of goiter in certain belts of our country and in Europe, in which there is a deficiency of iodin. In the United States the so-called goiter belt is about 200 miles wide and extends from "Old Smoky" in the southern Appalachian Mountains to the Canadian border in the Great Lakes region; it also includes part of the states of Washingon, Oregon, Idaho, and Montana. In the great Alpine valleys of Switzerland, France and Italy, and in other mountainous and glaciated districts of the Continent goiter is also prevalent. Not only human beings are affected in these regions, but animals, especially the domesticated animals. In the Great Lake regions many dogs are goitrous. In the region south of the Great Lakes the disease affects women more often than men, but, as the map on page 584 shows, males are goitrous in larger numbers in the Great Lakes region and in the Northwest. We shall return to this question in another Chapter,[2] where the different forms of goiter will be discussed.

Iodin and the Thyroid Gland.—Iodin was discovered in the thyroid gland in 1895, although it had been known for forty years that iodin therapy benefited goitrous subjects.[3] The average gland, when normal, contains about 0.05 g. of iodin per 100 gs. wet weight of substance. The content is higher in some animals than in others; the pig's gland contains 0.047 g., the cow's 0.036, and the sheep's 0.025. These are merely average figures, for there is a distinct seasonal variation, the content being about three times greater in the summer than in the winter. These variations have not been correlated with temperature, estrual periods, or other states, but undoubtedly some such factor is operative. The iodin seems to be associated with a

[1] Page 655.

[2] Page 654.

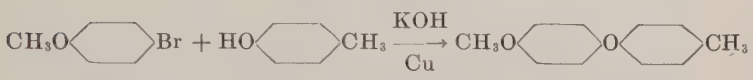
[3] For an account of the ravages of goiter in the Alps, and early attempts at treatment, see Whymper, E., Scrambles in the Alps, Philadelphia, J. B. Lippincott, 1873.

protid, known as thyreoglobulin. As we have shown before,[1] a definite compound, *thyroxin*, first isolated by Kendall and synthesized by Harington, probably represents the manner of association of the iodin in the gland. The molecule of thyroxin contains 65.3 per cent. iodin; whether the iodin or the nucleus is the effective agent has not been finally determined. Harington of the University College Hospital Medical School,[2] London, has been able to synthesize thyroxin as a derivative of tyrosin (page 271). This procedure was as follows:

(1) Preparation (as control) of des-iodothyroxin by removing iodin by means of KOH catalyzed by palladium calcium hydroxid.

(2) Synthesis of this iodin-free tyrosin, desiodothyroxin:

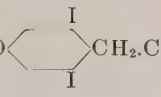
(*a*) Starting with toluene (p. 235), the methyl phenyl ether is bromated and conjugated with para-cresol:



(*b*) Through successive syntheses, through stages of hydantoïns (page 282), a substance having the properties of desiodothyroxin was obtained, the formula for both the natural and synthetic substance being:

Desiodothyroxion.

β-[4-(4-hydroxyphenoxy) phenyl] alanin.

Harington and the pharmacologist Barger succeeded in introducing iodin into this molecule. The resemblance of the substance to a compound gorgoïc acid (3, 5-di-iodo-tyrosin 

COOH) bearing two atoms of iodin in each aromatic ring probably best defines the composition of thyroxin. Kendall has described thyroxin as a "catalyzer of a catalyzer" (personal communication). That tyrosin is the basis of thyroxin is proven by Dakin who obtains tyrosin from thyroxin. There is a point of saturation of the gland which varies with the condition of the subject, etc. When iodin is administered saturation is complete; this amounts to about 0.005 g. of iodin per 100 gs. tissue, but it may be three times that figure. The

[1] Page 141.

[2] Harington, C. R. and Barger, G., Chemistry of thyroxine. III. Constitution and synthesis of thyroxine. Biochem. Journ., vol. 21, page 169, 1927.

action of thyroxin is probably catalytic, much as iron acts as a catalyzer—peroxidase in hemoglobin.[1]

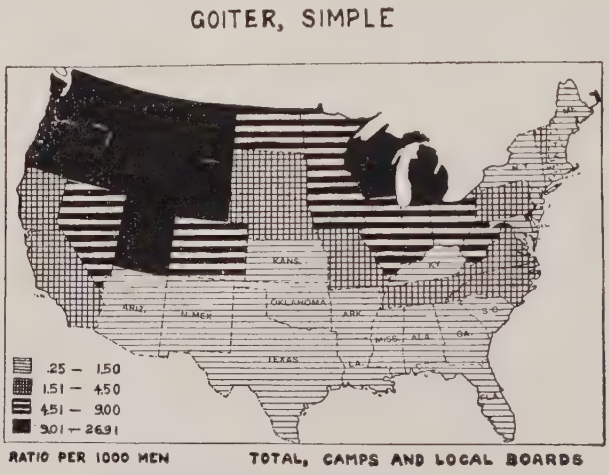


Fig. 174.—Comparative incidence of goiter, simple and exophthalmic, in the various regions of the United States. (Love, Albert G., and Davenport, Charles B., Defects Found in Drafted Men, 1920, p. 86. Reproduced by permission of the Surgeon-general.)

Iodin in Other Tissues.—Concerning the distribution of iodin in other tissues little is definitely known except that it is present in extremely small quantities. Consequently, nothing is known of the part iodin plays in the economy of the body as a whole. Iodin is

[1] For an account of Kendall's work see Ann. Clin. Med., vol. 1, p. 256, 1923. See also Fig. 53, p. 99, Applied Biochemistry.

not admitted to the aromatic ring unless an hydroxyl or other radicle is present. Thus, iodin forms 3, 5-di-iodo-tyrosin (gorgoïc acid)[1] when tyrosin is treated with iodin under special conditions; but phenylalanin, which lacks the hydroxyl or other radicle, does not hold iodin directly. The process of methylation is interfered with in thyroidectomized animals so that creatin is not formed,[2] but on feeding iodin, or thyroid substance, the power is restored.

The *utilization of iodin in the body* is modified by certain conditions. McCarrison[3] has found that experimental animals (pigeons) when subjected to filthy living conditions in soiled cages exhibit hyperplasia[4] of the thyroid gland, which is characteristic of iodin deficiency. The supply of iodin in the food was known to be adequate. The same animals, when fed on a diet excessive in oils and fats, exhibited similar hyperplasia. Therefore, McCarrison attributes the condition to an alimentary infection or to some other factor which inhibits the normal absorption of iodin. Somewhat similar conditions appeared during and at the close of the Great War in Europe, and in Asia among the human inhabitants of war-stricken localities. For some unknown reason the feeding of cod-liver oil to the animals living in foul cages prevents the hyperplasia mentioned above. The remedial effect may be due to absorption of iodin by the oil. Butter does not produce the same beneficial effect and the reason may be due to the fact that it has a lower iodin number and hence less power of carrying iodin than cod-liver oil.[5] It is possible that the vitamin content may have something to do with the increased utilization of inorganic substances in the body, for we know that calcium is utilized to better advantage when the body is treated with ultraviolet light or with irradiated foods.

To summarize: (1) Normally the largest quantities of iodin in the body are found in the thyroid gland. (2) The action of the iodin is probably that of an inorganic catalyzer. (3) It is associated in the gland with a protid. (4) Specifically, it is correlated with the normal or abnormal functioning of the thyroid gland.

For further considerations refer to the discussion of goiter, page 654.

[1] This is the basis of Harington's synthesis of thyroxin. See page 583.

[2] Page 364. The formation of creatin (methyl-guanidin-acetic acid) involves methylation.

[3] McCarrison, R. (Calcutta, India), Indian Jour. Med. Res., vol. 2, p. 1, 1923. See abstract in Jour. Amer. Med. Assoc., vol. 81, p. 1725, 1923.

[4] Greek *hyper*, above, and *plasis*, formation; *i. e.*, excessive growth.

[5] Iodin number of butter, 32; iodin number of cod-liver oil, 145.

Iodin as a Therapeutic Agent.—Boothby and Sandiford (page 656) in the United States and Harington and Dale in England have shown the quantitative relation between thyroxin and metabolism. Harington administered a decigram of thyroxin obtained by his method[1] to a normal person with B. M. R. −5 per cent. (see page 623). At the end of 48 hrs., the B. M. R., or metabolism, began to increase. On the 5th day, it had reached 40 per cent. The pulse rate had increased from the normal 72 per minute to 120. Harington's thyroxin, therefore, increases metabolism about 5 per cent. Iodides are administered in the treatment of various diseases, notably chorea (St. Vitus' Dance), arteriosclerosis, and other circulatory diseases and infections such as syphilis. An iodin rash frequently results from such treatment; the exact cause of this is unknown. We know, however, that iodin changes the nature of protid with which it is associated. When protid is iodized the body treats it as a foreign protid, and if the body has been sensitized[2] to such protid, reinjection causes marked specific response. It is possible, therefore, that the administration of iodin causes a change in the nature of protid and that the rash is due to the attempt of the body to deal with this foreign substance.

Substances Present in Minute Quantities.—On page 139, we have named many elements other than the ones just considered, as occurring in the body. Bertrand (page 349) has studied the effects of minute traces of inorganic substances, like manganese, nickel, cobalt, etc. On page 511, a discussion of the rôle of nickel and cobalt in sugar metabolism is given. Further research may show an even greater participation of small quantities of substances in vital phenomena. The recent discovery by Macallum of Johns Hopkins Medical School[3] of the immediate relation of iron in ossification is a case in point (page 358).

ACID-BASE EQUILIBRIUM IN THE BODY

In any study of nutrition the value of foods as acid or base producers is important. For example, herbivores ordinarily excrete a preponderance of bases and carnivores of acids. Metabolism is not affected by acid-base balance in health,[4] but is changed in disease

[1] The chief difference between Kendall's and Harington's methods consists in the substitution of dilute barium hydroxid for Kendall's NaOH.

[2] Page 325.

[3] Presented to the National Academy of Science, Philadelphia Meeting, 1926.

[4] Greenwald, I. (Roosevelt Hospital, New York), in Endocrinology and Metabolism, New York, D. Appleton & Co., 1922.

where the balance is disturbed in many cases. Modern biochemistry considers the mineral substances of the food in two ways:

1. As specific factors in the economy of the body, *e. g.*, iron in gaseous exchange in the blood, salt in holding globulin in solution, etc.

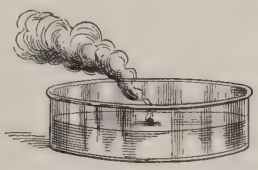


Fig. 175.—Potassium decomposing water. Potassium forms KOH, an alkali. This is readily determined by placing a strip of red litmus-paper in the water. Wood ashes and plant food ash in general contain K, and are basic. This is the basis of the dietary principle of increasing bases in the body by feeding vegetables. (From Holland, Medical Chemistry and Toxicology.)

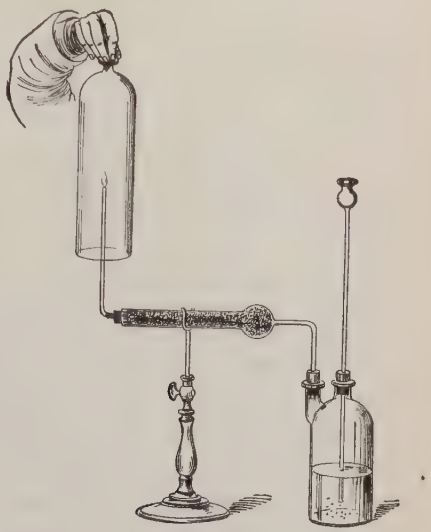


Fig. 176.—Water formed by burning hydrogen. All organic substances in the body contain hydrogen, which is converted into water during metabolism. The acidity of body fluids is due to ionic hydrogen. (From Holland, Medical Chemistry and Toxicology.)

2. As participating in the acid-base metabolism of the body. It is the latter aspect which we are about to consider.

The **acid**[1] **value of food in general** has to do with the concentration and behavior of *anions*, especially Cl^-, $PO_4^=$, $SO_4^=$. The base value of food in general has to do with the concentration and behavior of the *cations*, especially Na^+, K^+, Ca^{++}, and Mg^{++}. The balance of acid and base in the body is expressed wherever possible as base-balance. Thus:

Retention of base means positive (+) base-balance.
Retention of acid means negative (−) base-balance.
Acid excretion over intake means (+) base-balance.
Base excretion over intake means (−) base-balance.

[1] The "acid" is not that of moment acidity, that is, the *p*H, but the acid capacity of the substances concerned.

The **base-balance** is expressed as the number of mls. of 0.1 normal base. Sherman has determined the mineral content of the average diet of the people of the United States in terms of the acid-base values per twenty-four hours.[1]

Mineral Content of the Average Diet

Acid.	Mls. 0.1 normal.	Base.	Mls. 0.1 normal.
Phosphorus	925	Calcium	365
Chlorin	810	Magnesium	283
Sulphur	813	Potassium	870
		Sodium	850
Total	2548	Total	2368

2548 mls. decinormal acid
2368 " " base
180 " " acid[1]

[1] Table from Shohl modified from Sherman. See Physiol. Revs., vol. 3, p. 509, 1923.

The average person in the United States receives about 150 *mls. of acid per day;* in terms of base, man has a daily negative base requirement of about 150 mls. decinormal. We must distinguish this requirement from the balance, which is regulated by the excretion, (*a*) through the urine and (*b*) through the feces. The urine is normally acid[2] and the acid-base balance in the body determines how much acid is to be excreted through the urine. In the winter the diet consists largely of meat and other acid-producing substances, while in the summer, with the higher proportion of vegetables (base formers), a tendency toward a lower intake of acid-forming foods occurs. The balance, however, winter and summer is maintained by regulating the excretion and it shifts even during short intervals. Thus the "alkaline tide" following meals is due to the utilization of gastric juice acid, HCl, in the process of digestion, leaving a positive base-balance. Alkalosis develops when breathing is stimulated, as in mountain climbing, during which there is an "Auspumpung," as German investigators call it, a "pumping out" of CO_2, leaving a positive base balance in the body. All of these conditions are rapidly regulated by excess excretion of the appropriate materials. Thus, if CO_2 tends to increase in the body, the ventilating process of the lungs is increased by stimulation of the respiratory center, by the H^+ involved; ($CO_2 + H_2O = H_2CO_3$; $H^+ + HCO_3^-$). If alkali increases, there is an attendant increase in the excretion of alkaline substances in the urine, stimulated largely by the increase in osmotic pressure of

[1] Leaving out of account the mineral metabolism of iodin, manganese, arsenic, silicon, iron, etc., which exist in such small amounts that their acid-base value is negligible.

[2] Page 685.

the blood and tissues when alkali is retained. In addition to the regulatory power of the urine, the intestinal wall exhibits "selective permeability" upon mineral substances and, although this has not been definitely correlated with acid-base balance, there is an evident relation. As occasion demands, the intestinal wall permits mineral salts to leave the blood and to be excreted in the feces or to be absorbed from the intestine into the blood.[1] The mechanism of the alkali reserve, discussed earlier in the present volume,[2] is, of course, the immediate scene of equilibration of acid and base in the blood.

In **fasting** there is a greater excretion of acid substances (anions) than of base, due to tissue destruction freeing anions, like phosphates and sulphates[3] from the protids. A positive base-balance amounting to about 428 mls. decinormal base each day is thereby produced. Recovery from fasting is hastened on a diet preponderantly protid (acid formers), owing to the fact that there is a positive base-balance per day of 428 mls. of decinormal base which must be compensated for.[4]

In **pregnancy** there is a negative balance of about 67 mls. of decinormal base each day and this must be supplied by the mother to the fetus; this is probably the cause of that acidosis[5] which occurs in pregnancy, especially in later stages. Consequently, the pregnant mother should receive at least 70 mls. of decinormal base and preferably a "luxury" amount up to 150 mls. 0.1 normal base per twenty-four hours. After birth, the child retains base up to one year to the extent of about 12 mls. per kilogram of body weight. It is used largely in making of bone, but also of protid.[6] Calculated out in terms of decinormal solution all the base retained by a growing child is distributed as follows:

TABLE SHOWING BASE RETAINED BY GROWING CHILD

	Mls.
Protid retained per day as decinormal base	2
Alkali reserve	4
Bone	50
Unaccounted for	2
Total base retained	58

[1] Goldschmidt, S. (University of Pennsylvania, Philadelphia), Physiol. Revs., vol. 1, p. 421, 1921. [2] Page 83.

[3] Actually, phosphoric acid, and sulphur in the form of SH sulphur freed from the molecule and oxidized in the liver to sulphuric acid, etc. See page 794.

[4] Deprivation of glucids leads to acidosis. In fasting, this factor, which depends upon the degree of starvation, is encountered.

[5] Loss of base means that base must be supplied. The bases are retained in the body of the fetus and used for building the body in growth of the bones, etc.

[6] About 0.7 g. per twenty-four hours. Protid is made to the extent of about 3 gs. daily.

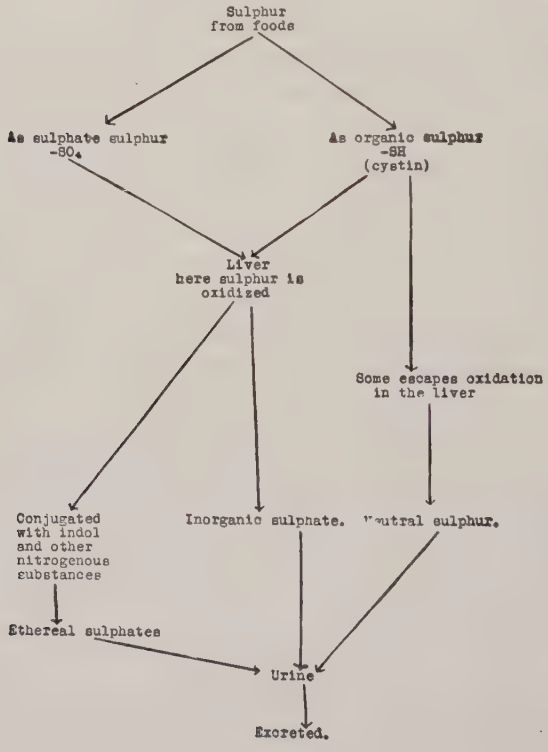
The necessity, therefore, of supplying adequate amounts of base-forming substances to a mother with child or to the child itself during growth, is evident.

The Regulation of Acid-base Equilibrium in the Body.— This is largely a matter of the excretion of acid or of base substances in (*a*) the urine, or (*b*) feces.

The *urine*, as we have said, is normally acid, depending upon:

1. The phosphate NaH_2PO_4.
2. Urea, representing conjugated cyanic acid and ammonia.
3. Ethereal sulphates, etc., representing inorganic acids neutralized by conjugated radicles.
4. Organic acids, like oxalic.
5. Carbonic, as bicarbonate and carbonate, $NaHCO_3$ and Na_2CO_3.

GRAPHIC SUMMARY



The *feces* are usually alkaline by about 250 mls. of decinormal base. Some of this basicity is due to the mineral substances in the undigested foods, but some comes from the bases excreted from the blood into the intestine. The part played by the feces, therefore, is

the opposite of that of the urine, and we may say that urine lowers the acid-producing substances, while feces lower the basic radicles.[1]

Acids administered for any purpose, such as HCl to increase the concentration of gastric acidity in hypo-acidity, cause excretion of alkali in two general ways:

(1) Loss of alkali minerals partly by the kidney and in part by the feces.

(2) Loss of ammonia quite entirely through the urine. Alkali when given for hyperacidity of the gastric juice, in acidosis when sodium bicarbonate is given, and in other cases is retained to some extent, and if there is enough alkali retained, there is a consequent absorption of water by the body. The effect of administering alkali is thus summed up by Shohl:

In the urine: (1) Urine acidity decreases equivalent to 200 mls. 0.1 normal acid.

(2) Low ammonia in the urine, or none at all.

(3) Phosphates in the urine become alkaline phosphate, B_2HPO_4.

(4) Neutralization of organic acids.

(5) Increase in carbonate.

In the feces: Increased excretion of alkali in the form of calcium soaps, calcium phosphate, and carbonate.

The following tables show the reaction of fluids used as food and occurring in the body (Clark):

Table Showing Reaction of Fluids

	*p*H.		*p*H.
Blood	7.3	Aqueous humor	7.1
Urine (average)	6.0	Vitreous humor	7.0
Saliva (37° C.)	6.9	Cerebrospinal fluid	7.2
Gastric juice (ad.)	0.9–1.6	Amniotic fluid	8.1
Gastric juice (juv.)	5.0	Milk (human)	7.0–7.2
Pancreatic juice (dog)	8.3	Milk (cow)	6.6–6.8
Intestinal (small) juice	8.3	Milk (goat)	6.6
Same for infant	3.1	Milk (ass)	7.6
Bile (liver)	7.8	Sea water	8.0
Bile (cyst)	5.3–7.4	Mineral waters	6.5–7.0
Perspiration	4.5	Flour (extract)	6.0–6.5
Tears	7.2	Maple syrup	6.8
Muscle, expressed	6.8	Vinegar	3.1
Pancreas, expressed	5.6	Vinegar, synthetic	2.6
Peritoneal fluid	7.4	Feces (adult)	7.1–8.8
Pericardial fluid	7.4	Feces (juvenile)	6.0–7.0

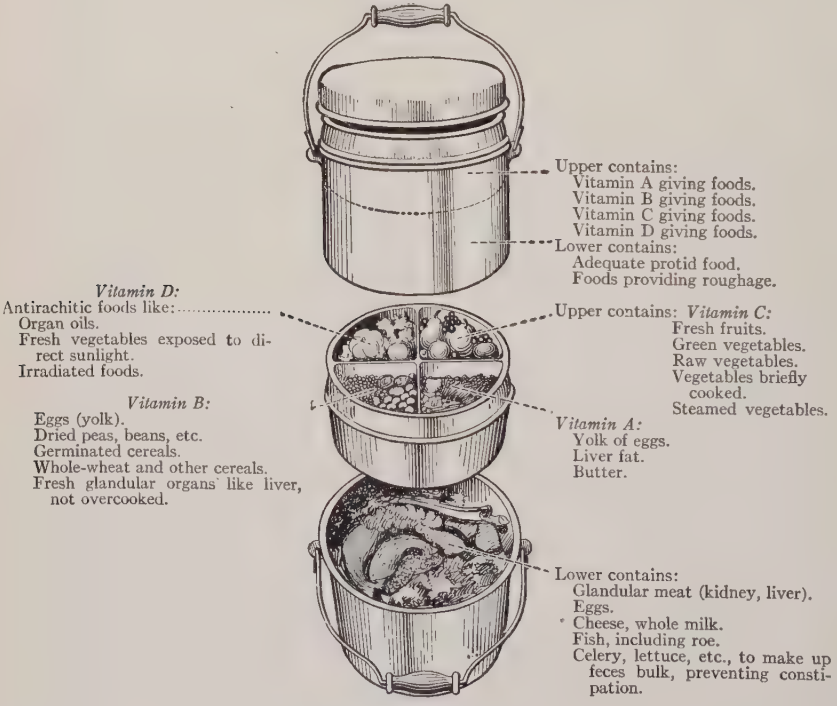
[1] The average recretion of normal stools is *p*H 6.6. Purgation causes increased acidity. Thus NaH_2PO_4, Carlsbad Salts (mixture of Na_2SO_4, K_2SO_4, NaCl and Na_2CO_3), rhubarb, etc. give stools of *p*H 5.16 to 6.85. One favorable effect of purgation may be due to change in reaction of stools with accompanying change in intestinal flora.

Table Showing the True Reaction of Fruits

	*p*H.		*p*H.
Lime juice	1.7	Grape-juice	4.5
Lemon	2.2	Prune juice heated	4.3
Cherry juice	2.5	Apple juice heated	3.8
Grapefruit	3.0	Banana juice heated	4.6
Orange	3.1	Bean (string)	5.2
Rhubarb	3.1	Carrot juice heated	5.2
Strawberry	3.4	Cucumber juice heated	5.1
Pineapple	3.4	Beet juice heated	6.1
Tomato	4.2	Potato juice heated	6.1
Pear	4.2		

Summarizing we may say: (1) Acid causes a negative base-balance. (2) Alkalis cause positive base balance or an increase of a positive base-balance.

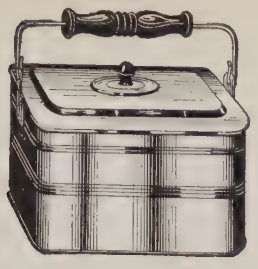
Normal and Abnormal Diets.—By means of Figs. 177 and 178 it is hoped that the reader may visualize a balanced ration and be



The full dinner-pail.

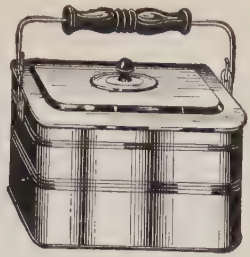
Fig. 177.—A normal diet.

able to appreciate an abnormal diet. It is supposed that the diet has been devised for an average workman who demands about 3000



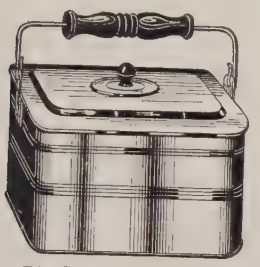
Diet A Lacks Vitamin A:

Contains:
- Vegetable oils ("vegetable lard," etc.).
- Oleomargin and butter substitutes.
- Animal lard.
- Bacon fat.
- Fat-of-pork ("salt pork").



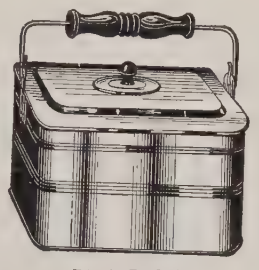
Diet B Lacks Vitamin B:

Contains:
- Rolled oats and other degerminated and decorticated grains.
- Muscle meats.
- Salted (brine) meats.
- Overcooked vegetables, meats, etc.
- Excess sugar.



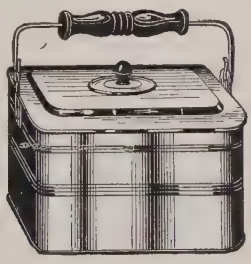
Diet C Lacks Vitamin C:

Contains:
- Boiled vegetables.
- No fresh fruits.
- Only muscle meats.
- Autoclaved or overcooked vegetables and meats.
- Devitaminized milk.
- Fruit juices that have been heated in canning or bottling or have lost their vitamin on standing.
- Sterilized foods.



Diet D Rachitic:

Contains:
- Highly milled cereals.
- Foods grown in the absence of sunlight.
- Overamount of starchy foods lacking protid, like white bread.



Diet E Lacks Complete Protids and Roughage:

Contains:
- Gelatin (as puddings, etc.).
- Corn (maize), decorticated and highly milled.
- Wheat and other cereal preparations of highly milled flours.
- Readily digested substances leaving no residue.
- Steak meats, both fish-steaks and meat, as steaks, chops, etc.

Fig. 178.—Abnormal diets.

Cals. (see page 629). The problem is to afford sufficient nutriment for maintenance. In the nature of the case it is impossible to erect a single graphic representation applicable to growth, maintenance, etc. The series of diets represented in Fig. 178 are incomplete in certain regards, the deficiencies being either vitamin (first four) or incomplete nitrogenous or roughage substances.

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CHAPTER XIII

THE ENERGETICS OF NUTRITION

"By metabolism is meant the chemical changes of materials under the influence of living cells."—*Lusk.*

In the preceding Chapter we have studied the chemical constituents which make up the body. The question arises, Why are these materials constantly undergoing change and being broken down to substances which are excreted from the body? The answer is that the body is not a static thing, but, as Herbert Spencer[1] said, "Life is a continual adjustment of internal to external conditions." Since external conditions change continually, there must be corresponding changes within the organism. For example, in the maintenance of temperature a continual adjustment of internal conditions to external is necessary. The promotors of chemical action, the enzymes, act at certain temperatures better than at others, and some means must be provided to maintain the optimal temperature. This is accomplished by introducing oxygen to cause combustion of substances in the body, sugars and protids. We breathe air for this reason.

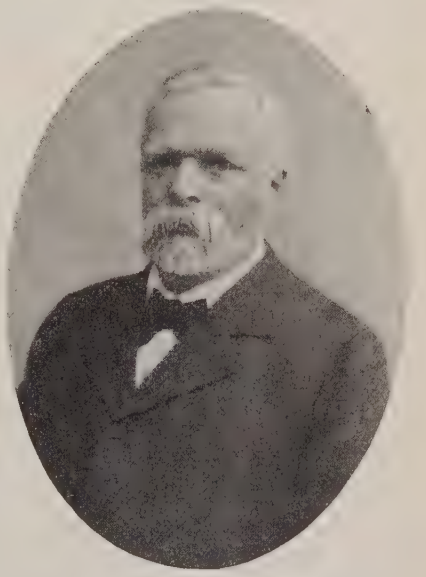


Fig. 179.—Carl Voit, Physiologist, Physiological Institute, University of Munich, Bavaria. Responsible for much of our knowledge of the energetics of metabolism and for the beginnings of American studies in that field, carried further by Atwater, Lusk, and their colleagues.

Forms of Energy in the Body.—There are various forms of energy in the body. We have seen that movement is accomplished by special-

[1] Spencer, Herbert, English philosopher of the 19th century. See his Principles of Biology, vol. 1, New York, D. Appleton & Co., 1898.

ized tissues, the muscles, and that the cause of the contraction of muscle is the swelling of the fibers due to the absorption of water during the time when their membranes are rendered more permeable to water by lactic acid formed from glucose. Likewise, there is a parallel or subsequent combustion of some of the sugar or acid which produces heat to maintain the proper temperature for the reaction. It is granted that a secreting gland does work; then the same may be said of the cells of the intestine where absorption of digested foods is taking place. Even a nerve performs work, as Tashiro[1] has shown, the exact nature of which, to be sure, we do not know.

The Principle of Joule.—The desirability of a single standard, whereby all types of energy changes within the body might be expressed quantitatively, is evident. Thanks to an English physicist, Joule,[2] this is possible.

Heat unit	=	gravity units	=	electrical units	=	work unit	=	gas-pressure unit
1 cal.[3]		42,600 g. cms.		116,200 watt-hours.[4]		41,860,000 ergs.		$\frac{1}{41,830}$ liter atmospheres.

= mechanical equivalent of heat 4.186 joules.

All forms of energy must be expressed in terms of heat units. In the human body all forms of energy seem to be eventually converted into heat, the lowest form of energy. The intake of energy expressed in units of heat calories balances the outgo of energy expressed in similar terms. The body energy is "degraded" into heat, a form of energy of low vibration and of long wave-length.

Efficiency of the Human Machine.—Measured in terms of heat evolved, the greatest amount of energy a man may produce in one hour is about 600 Cals.[5] This would mean 33 per cent. efficiency. However, only a portion of the whole energy of the body is derived from combustion and, therefore, it is not possible to compare the human mechanism with a combustion engine or to express the efficiency of the human organism definitely. Muscle, according to

[1] Tashiro, Shiro (University of Cincinnati), A Clinical Sign of Life, University of Chicago Press, 1919.

[2] Joule, J. P., British physicist of the middle of the 19th century.

[3] Small calorie, not the one used in the energetics of human metabolism, which is one thousand times this.

[4] One one-thousandth of the unit employed in estimating house-currents; the unit there is the kilowatt.

[5] Henderson (Henderson, Y., and Haggard, H. W., Jour. Biol. Chem., vol. 63, p. lxix, 1925) finds the maximal energy expenditure in man is from thirteen to twenty times the B. M. R., or about 1700 Cals. per hour.

Hill,[1] is about 20 per cent. efficient. Muscle energy is about half the total body energy, a relatively large amount being derived from other sources, such as osmosis, absorption, etc.

Entropy and the Limitation of Efficiency.—In all interconversions of energy, some becomes bound. This bound energy is known as entropy, which may be defined as non-productive energy. The physicist has shown that since all energy manifestations involve the formation of bound energy, the universe must be running down. He says that "entropy tends to a maximum." This statement is applied only to closed systems and nothing is said concerning the influence of one system on another. In the case of the human being, all energy transformations are made within the cell, which is virtually a closed system. However, there are interactions of the cells and it may be that one characteristic of life is the ability of one system to affect another favorably.[2]

The Entropy of Carbon.—Carbon is the most characteristic element of the body. Undoubtedly, one reason for this is its unique ability to bind many atoms of the same kind. The entropy of carbon, moreover, is the lowest of the elements in the human body. The following table serves to compare the entropy of carbon with that of other elements:

ATOMIC ENTROPY OF SEVERAL ELEMENTS IN THE BODY[1]

Calcium	11.0	Magnesium	8.3
Carbon (diamond)	0.6	Nitrogen	22.8
Carbon (graphite)	1.3	Oxygen	24.1
Chlorin	25.7	Potassium	19.7
Hydrogen	15.9	Sodium	12.2
Iodin	15.7	Sulphur	7.6
Iron	6.6	Silicon	4.7[2]

[1] Derived from the expression: $S = \int \frac{d\theta}{T}$, where S is the entropy, $d\theta$ heat, which must be added to the system to maintain the temperature T (on the absolute scale); $\int$ the sign of summation of the calculus. Less technically, entropy $= \frac{\text{Heat units}}{\text{Absolute temperature}}$ units.

[2] It is interesting that silicon has a low entropy. It has been pointed out that the living world may have been built upon a silicon basis rather than a carbon one.

Carbon, with an entropy of 0.6, has energy efficiency greater than any element in the organism. How much this counts in estimating the efficiency of the organism is unknown, but it is certain that as a

[1] Page 376.

[2] For a modern treatment of entropy see the illuminating Silliman address of Lewis (page 35): The Anatomy of Science. New Haven, Yale University Press, 1926.

whole the efficiency of the body is enhanced by the use of such an element.

What relation has "energy" to medicine? The question may be asked, Of what especial value are such considerations to the physician? The question is answered as follows: Individuals differ in their expenditure of energy, and consequently, as to their food requirements. The physician must, therefore, have some means of designating the value of foods for given cases. Again, certain diseases cause an abnormal increase in the proportion and utilization of energy, while others cause a reduction. For example, exophthalmic goiter is characterized by increased energy production and expenditure, whereas myxedema involves an abnormal reduction.

CALORIES

The Calorie: The Unit of Energy in Biochemistry.—Since heat is the standard for comparing energy relations in biochemistry, the unit of heat has been adopted to express energy in concrete terms. This is known as the Calorie, and is defined as the amount of heat necessary to raise the temperature of 1000 mls. of water from 15° C. to 16° C. This is the large Calorie, composed of 1000 small calories, which are defined as the amount of heat necessary to raise the temperature of 1 ml. of water from 3.5° to 4.5° C., the temperature at which water has the greatest volume. The average daily requirement of a man is 3000 Calories; for a woman, 2575, or six-sevenths of that figure. The average man must, therefore, consume food which contains at least 3000 Cals., and a woman about 2600, every twenty-four hours. A boy of fifteen years requires about as many Calories as an adult man. These questions will be considered later.

Relations of Calories outside the Body to Those within.—Have we reason to believe that if the heat evolved by the burning of a given amount of food outside the body is determined, it will indicate the value of this food when consumed? The question was answered at the close of the eighteenth century in England by Crawford and in France by Lavoisier,[1] who found that the same amount of heat is given off from a known amount of carbon *in vitro* as *in vivo*, that is, the same number of calories of heat are given off by carbon burned as a candle in the open air as carbon burned in the body. Moreover, the Law of Hess[2] assures us that it is immaterial how the burning is

[1] For a historical introduction see Atwater, W. O. Articles in the Century Magazine for 1887 and later.

[2] The same amount of heat is evolved whether the burning is direct and immediate or through intermediate stages.

effected, whether by explosion or through a series of intermediate stages, the same amount of heat is produced. Hence, we may burn a sample of food under control in the laboratory and derive the number of calories which it will give as a minimum when utilized in the body. We say "as a minimum," because of the specific dynamic action discussed earlier[1] and other factors not well understood. There is 10 per cent. more heat produced by glucose when burned in the body than when it is oxidized in the laboratory.[2] The same is true of fat and protid to a greater extent. Levulose gives greater discrepancies. The following table gives the percentage increase in heat production by different glucids expressed in terms of the rise in metabolism over basal metabolic rate.[3] These figures are proportional to the differences the sugars show when burned in the laboratory and when burned in the body:

TABLE SHOWING INCREASE IN HEAT PRODUCTION WHEN SUGARS ARE FED OVER BASAL METABOLIC RATE (LUSK)

	Gs.	Percentage increase two hours after ingestion.
Glucose	50	30
Glucose	70	35
Fructose	50	37
Sucrose	50	34
Galactose	50	22
Lactose	50	3

The following table gives the actual fuel values:

Total nutritional value:

	Cals.
Glucids.—98 per cent. utilized:	
Theoretical yield, 4.1 Cals. per 1 g. glucid	
Actual (98 per cent. of 4.1)	4.0
Lipids.—95 per cent. utilized:	
Theoretical yield 9.45 Cals. per g.	
Actual (95 per cent. of 9.45)	8.975
Protids.—92 per cent. utilized:	
Theoretical yield 4.35 Cals.	
Actual (92 per cent. of 4.35)	4.125

Determination of Calories Outside the Body; Food Calories.—This is accomplished by burning a dried sample under controlled conditions in which the rise in temperature can be accurately determined

[1] Page 565. In addition, there is the fact that protid is not burned to products in which the greatest possible amount of oxygen is contained. See page 539.

[2] The extra heat is derived from substances other than glucose, glucose itself being able to produce only a certain amount of heat, indicated by the calories found when it is oxidized outside the body.

[3] For a definition of this term see page 622.

and the Calories calculated from such data. One of the commonest methods is the use of a bomb calorimeter, a heavy, hollow metal sphere in which the substance to be burned is ignited electrically in an atmosphere of oxygen. The heat produced by the oxidation is transmitted through the walls of the bomb to the surrounding water which is kept at known temperature in a thermostat. The rise in temperature of the water is recorded on a delicate thermometer. Knowing that a Calorie is the amount of heat necessary to raise the temperature of 1000 mls. of water 1° C., the proportionate rise from the burning of the substance may be readily determined. We give the details of the *Emerson bomb apparatus;* an excellent type is that of Lusk-Riche.[1]

The Construction of the Emerson Bomb Apparatus.—In order to isolate the heat produced by combustion, or other chemical change, from that of the surroundings, the combustion is done in a thermostat, or water-bath. This is simply a large metal vessel provided with a shiny exterior surface to reflect the heat rays from the outside and a layer of insulating material to avoid heat transfer from within, out, or vice versa. A cover is provided with holes for the thermometer, the wires going to the bomb and the thermoregulator. The bomb is made of steel and is composed of two hemispheres held together by heavy threaded screws. There are two connections for the wires, admitting the electric current used to explode the substances within the bomb. The bomb contains a platform for the small vessel designed to hold the material under examination. The lining of the bomb is platinum, palladium, or gold. The small vessel is also made of one of these materials. A side or top opening is arranged for admitting oxygen gas from a cylinder.

The Determination.—Obtain the constant for the particular calorimeter in use. The United States Bureau of Standards, Washington, D. C., supplies naphthalin, a coal-tar product, which burns with the evolution of a given amount of heat per known weight. This amount of heat is written on the container. The small, accurately weighed amount of naphthalin is placed in the small vessel referred to above and it is inserted into the bomb. The hemispheres are screwed together and tightened with a long-handled wrench, 1 meter long. The electric wires are placed in position in the holes provided for this purpose. Then oxygen under about 25 atmospheres pressure is admitted from the cylinder and the small aperture for the gas is closed securely. The bomb is lowered into the water in the thermo-

[1] Jour. Amer. Chem. Soc., vol. 35, p. 1747, 1913.

stat. After temperature equilibrium is reached the current switch is closed; a current is sent through the naphthalin resting within the bomb by means of a small iron wire which becomes heated or, better, a thread of fiber passing through the naphthalin joining the ends of

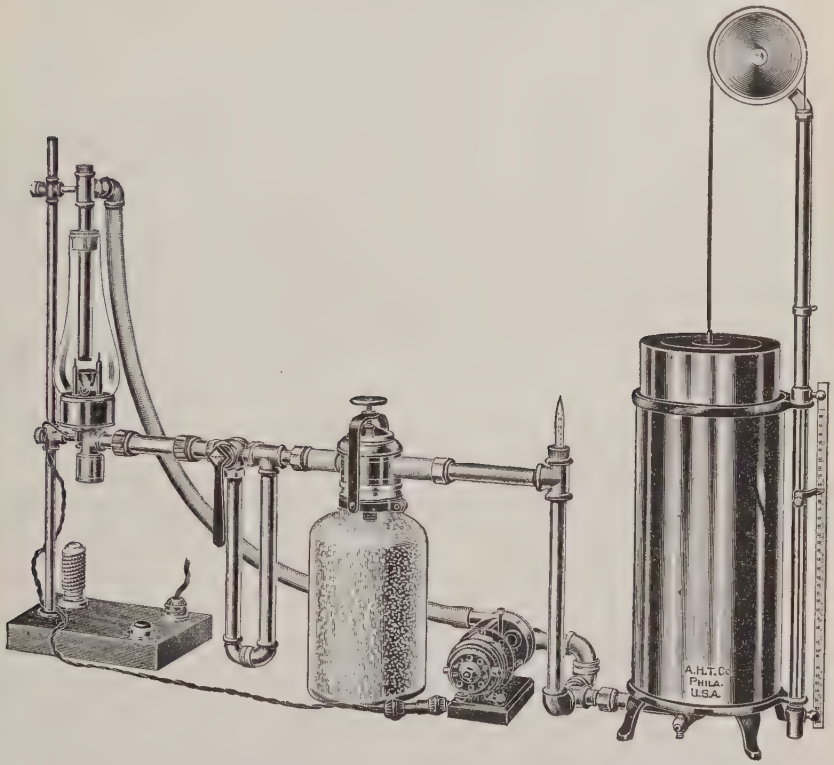


Fig. 180.—Oxy-calorimeter, F. G. Benedict. (Nutrition Laboratory, Carnegie Institution, Washington) for determining the heat of combustion of materials burned in the living organism.

The motor circulates the oxygen through the system, soda lime absorbs the CO_2; the sample is ignited electrically and burns as the oxygen circulates past it; the spirometer is filled with oxygen and measures the amount used. The computation consists simply in determining the amount of oxygen required for complete combustion and the application of the proper heat factor.[1]

the wires and completing the surface, becomes ignited. The high content of oxygen insures complete combustion; the heat liberated is transmitted to the water and the rise in temperature is noted upon a thermometer graduated in hundredths of a degree (Fig. 21). Such a

[1] Francis G. Benedict and Edward L. Fox, *Industrial and Engineering Chemistry*, Vol. 17, page 912, 1925, and *The Journal of Biological Chemistry*, Vol. 66, page 783, 1925.

thermometer as the Beckmann, used in freezing-point determinations,[1] serves for this purpose. This thermometer shows only about 6 degrees, so that the finer graduations of 1/100° C. are large enough to study with a magnifying glass, or even with the naked eye.

Having noted the change in temperature by this means, one may calculate the difference between the heat generated and the theoretical amount which may be calculated from the data on the bottle of the naphthalin. This discrepancy is the constant for the apparatus; the quantity must be deducted from the readings in all future determinations of foods. As an example we may take the following:

Heat of combustion of naphthalin, $C_{10}H_8$, 1084 small cals.
This means that 1 gram-molecule when completely oxidized gives 1084 small calories of heat.
Taken for the determination of the constant, 250 mgs. of $C_{10}H_8$.
Heat of combustion of 250 mgs. $C_{10}H_8$, 271 cals.
Heat of formation of CO_2 and H_2O, the products of combustion of

$$C_{10}H_8,\ 96.9 + 115.2 = 212.2 \text{ cals.}$$

Difference, being the actual calories expected, 58.8 cals.
Degrees Centigrade rise in temperature after explosion, 14.8° C.
This is equivalent to 59.2 calories, since a calorie is the amount of heat necessary to bring 1 g. 1 degree higher in temperature; $\frac{1}{4}$ gram (250 mgs.) was taken.
Therefore, the constant for the bomb in question is the difference between the expected and found, namely, 0.4 cal., which must be deducted from any other determination.

The Preparation of the Specimen for Combustion.—The specimen must be thoroughly dried, but not decomposed by heat. Drying at 40° C. in a current of air is customary. The specimen is then pulverized, made into pellets, or carefully weighed into the vessel.

The determination of the heat: This is done as described above for naphthalin.

Calculation: From the heat determined subtract the constant for the calorimeter and express the answer in terms of large Calories per 100 gs. of substance.

Calories in Common Foods.—In hospitals, restaurants, etc., attention is directed to the caloric value of foods served. The following table gives approximately[2] the Calories in some common foods.[3] For a complete list see Gephart:

[1] Page 50.

[2] The Calories, vitamin values, etc., given on many restaurant menus are frequently absurdly inaccurate.

[3] Benedict (F. G.) has recently designed an oxy-calorimeter (Fig. 180) which determines caloric values of foods, etc., by oxygen utilization.

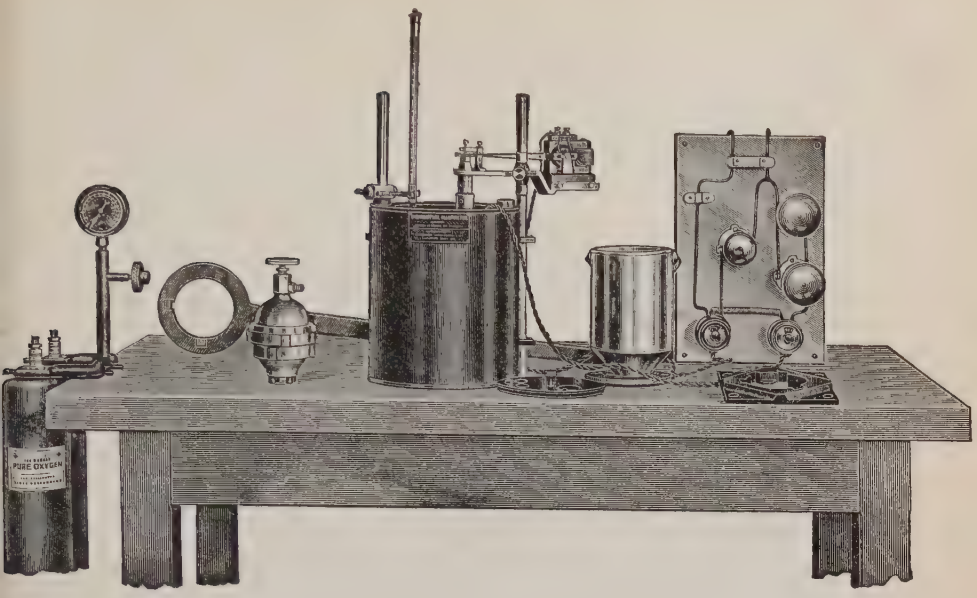


Fig. 181.—A common form of food-calorimeter. The Emerson Bomb Calorimeter. From left to right: Oxygen cylinder; gas-pressure indicator; wrench for closing bomb; bomb; thermostat, bearing thermometer and stirrer; support for bomb; lamps for regulating current used to explode the bomb.

Breakfast dishes

	Cals.
Shredded wheat with milk	180
Corn flakes with milk	120
Porridge (oatmeal) with milk	190
Boiled white rice with milk	100
Poached egg on toast, two eggs	300
Omelette, plain	200
Banana	100
Baked apple	100
Baked apple with cream	150
Stewed prunes	140
Apple sauce	80
Sliced orange	100
Coffee, per cup, black (tea, postum, etc., identical)	8
Cocoa ("hot chocolate"), plain	200
Milk, glass	200
Buttermilk	100
Bean soup, 240 mls.	250
Chicken and rice soup	250
Cream of tomato soup	250
Vegetable soup	225

Lunch dishes

	Cals.
Biscuit, baking powder, 90 gs	200
Bread, wheat, white, 90 gs. (also graham; bran; rye, etc.)	150
Coffee cake	242
Doughnuts	400
Corn muffins	250
Baked codfish	200
Codfish cakes	250
Filet of flounder, fried	225
Boiled halibut steak	200
Fried oysters	135
Oyster stew	250
Eggs, boiled, two	150
Bacon, broiled	300
Corn beef hash	325
Fried ham	400
Ham and eggs	390
Small steak	300
Baked beans	350
Spaghetti and cheese	240
Chicken croquette, cream sauce, and potatoes	250

Dinner dishes

	Cals.
Sausage	300
Sausage and buckwheat cakes	450
Creamed asparagus	125
Beets, plain	50
Cabbage, boiled	60
Carrots, buttered	85
Cauliflower, buttered	90
Fried egg-plant	120
Green peas, buttered	230
Lima beans, buttered	200
Onions, buttered	150
Potatoes, baked	200
Spinach, buttered	85
Tomatoes, stewed	80
Lettuce and tomato salad	50
Potato salad	70
Sliced tomatoes, plain	35
Club sandwich	600
Cantaloupe	50
Cup custard	150
Ice-cream	225
Grape fruit, half	70
Orange juice	70
Apple pie, per cut	280
Mince pie	400
Cake, plain	200

Other factors being equal, one may arrange a diet having a certain caloric value by taking isodynamic equivalents of foods, that is, having the same heat value. The reader is warned at once, however, that the total value of any food cannot be measured in Calories. Many other considerations enter into such evaluation. The thermal value alone is indicated by Calories. Foods are therefore seldom "isodynamic."

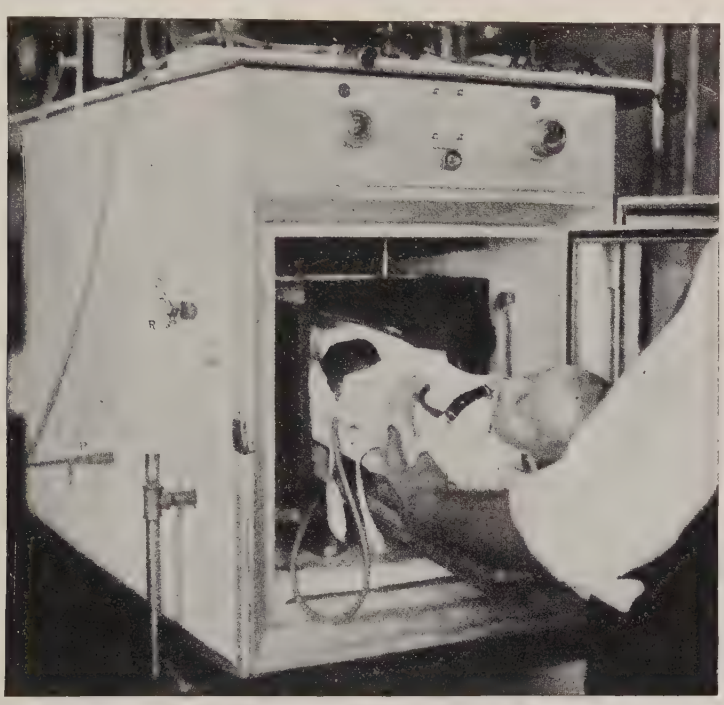


Fig. 182.—Direct calorimetry. Metabolism chamber for estimating the calories of energy utilized by a child. The condition of the subject is carefully watched by means of the leg-cuff (for pulse) and chest pneumograph (for respirations). For plan of a room calorimeter, see page 607, Fig. 183. (Murlin and Hoobler, Amer. Jour. Dis. of Child., vol. 9, p. 89 (Fig. 1), 1915.)

Calories Within the Body.—How can the number of Calories of energy required by a human being be critically determined as a basis for scientific feeding? The appetite cannot be trusted, because one individual may eat more than he requires, while another may not eat enough. Moreover, in great crises, as in war time, how can food be scientifically apportioned among the populace? The rations of the U. S. Army are given here in terms of Calories. An instance of sad misjudgment was given during the Great War when, in the very city where much of our knowledge of nutrition was derived, the

children were rationed far below their needs. The graph on page 624 shows the number of Calories required by boys.

Caloric need of U. S Army soldiers:

Mechanical efficiency, 33 per cent.

Load	Road	Normal	Needs Cals. Extra	Total	Waste
None	Level	2064	+900	2964	3260
50 lbs.	"	2064	1172	3236	3560
"	Ascending 100 ft. per mile	2064	2110	4174	4590

Rations

	Cals.
(1) For intensive work (entrenching, etc.)	5000
(2) General routine	4000
(3) Mobile, minimum transport	3500
(4) Iron ration (see below)	2825

Iron ration

Corned beef	7.0 oz.	210	C.
Pork and beans	8.5 "	255	"
Bread (Hard tack)	6.6 "	198	"
Chocolate	3.0 "	90	"
Coffee	1.80 "		
Sugar	2.40 "	0.60	"
	per day.	70.0	

Methods of Calorimetric Determination.— Two chief methods are in use: (1) Physical, or direct method, and (2) Chemical or indirect.

Direct Method of Calorimetric Determination.—The principle of this method is the same as that described on page 599 for finding food calories, *i. e.*, causing combustion within a space insulated from without. In the case of the food calorimeter, a bomb was placed in a thermostat. The material being analyzed was placed in the bomb. In human calorimetry the bomb is replaced by the human body and a compartment large enough to hold the body, or larger, replaces the thermostat. A certain amount of movement during the studies of metabolism of exercise, etc., is necessary. For the details of such a calorimeter see Mathews' Physiological Chemistry. Briefly, the principal factors are as follows: The absorption of the heat derived from the subject is effected by ice-water (constant temperature) circulating through pipes suspended in the calorimeter. The amount of water passing through it for a given length of time is determined by weighing the water after it leaves the chamber. The walls of the

calorimeter, being hollow, insulate the chamber from the outside, and hence all thermal changes are recorded by the difference in temperature of the water as it enters and leaves the chamber. The room is ventilated by a pipe system and rotary blower and the air is passed through absorbers (H_2SO_4 and moist soda lime) to remove the CO_2 and H_2O. By weighing the absorbers, before and after a run, the

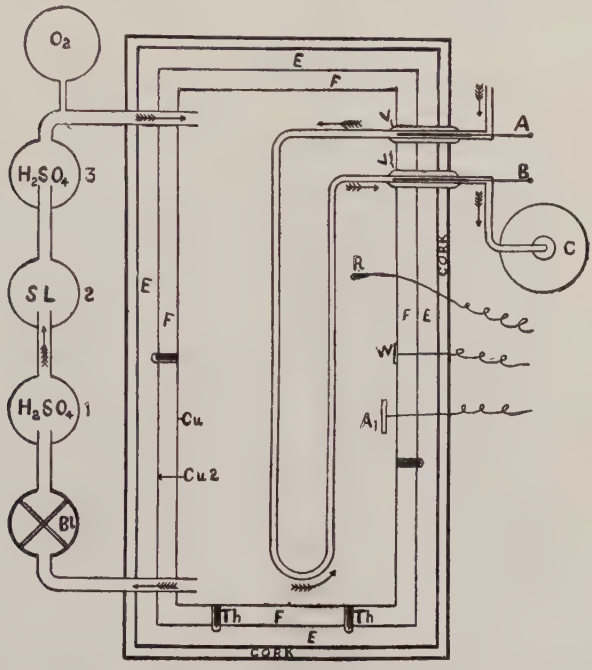


Fig. 183.—Schematic diagram of the Atwater-Rosa-Benedict respiration calorimeter (From Lusk, Elements of the Science of Nutrition.)

Ventilating system: 1, H_2SO_4 to remove moisture given off by patient. 2, Soda lime to remove CO_2. 3, H_2SO_4 to catch moisture given off by soda lime. O_2, Oxygen introduced as needed by subject. *Bl*, Blower to keep air in circulation.

Indirect calorimetry: Increase in weight of H_2SO_4 (1) = water elimination of subject. Increase in weight of soda lime (2) + increase in weight of H_2SO_4 (3) = CO_2 elimination. Decrease in weight of oxygen tank = oxygen consumption of subject.

Heat-absorbing system: *A*, Thermometer to record temperature of ingoing water. *B*, Thermometer to record temperature of outgoing water. *V*, Vacuum jacket. *C*, Tank for weighing water which has passed through calorimeter each hour. *W*, Thermometer for measuring temperature of wall. A_1, Thermometer for measuring temperature of the air. *R*, Rectal thermometer for measuring temperature of subject.

Direct calorimetry: Average difference of *A* and *B* × liters of water + (gs. water eliminated × 0.586) ± (change in temperature of wall × hydrothermal equivalent of box) ± (change of temperature of body × hydrothermal equivalent of body) = total Calories produced.

Th, thermocouple; *Cu*, inner copper wall; Cu_2, outer copper wall; *E*, *F*, dead air-spaces.

amount of these gases removed from the air of the chamber for a given time is determined. Oxygen is continually fed into the chambers in order to replace that used by the subject. The direct determination of heat is made by multiplying the volume of water passed through the apparatus for a given period by the temperature difference of the water before and after passage through the calorimeter; this makes up about 75 per cent. of the heat given off by the subject. The remaining 25 per cent. of the heat from the subject within the calorimeter is caught as latent heat in the water-vapor absorbed by the H_2SO_4; about half a Calorie of heat is contained in each gram of water-vapor.[1] This must be added to the heat found in the circulating water, and the two quantities constitute all of the heat derived from the subject in the calorimeter during the run. The results are expressed in Calories per hour.[2]

Indirect Method for Calorimetry.—Principle: A man utilizes about 1 liter of oxygen for every 5 Calories of heat given off; this has been determined by direct calorimetry. However, within narrow limits, the caloric value of one liter of oxygen varies according to the character of the food; on a diet of fat, 4.686 Cals. are given off from the body for each liter of oxygen consumed, while on a diet of starch, sugar, etc., each liter of oxygen corresponds to 5.047 Cals. A protid diet gives an intermediate figure. It has been found by French investigators of the last century that the amount of CO_2 given off by a carnivorous animal (dog) compared with that given off by a herbivore (rabbit) is less, proportionate to the oxygen consumed; that is, $\frac{CO_2}{O_2}$ for a meat-eating animal is less than the ratio for a vegetable-eating animal. The character of the food determines the caloric value of oxygen as well as the value of the ratio, known as the "respiration ratio," or quotient, $\frac{CO_2}{O_2}$. Hence, by determining the

[1] The amount of water-vapor in the air cannot be judged by fog or steam. Few persons would believe that there are only 30 mls. of liquid water in enough atmospheric fog to fill the volume of a common freight car, yet that is the usual amount as determined by measurements made by one of the United States Government departments.

[2] In the United States, direct calorimetry for the human subject is possible in the following laboratories: Washington, D. C., Department of Agriculture; Nutrition Laboratory, Boston, Mass.; Carnegie Institution of Washington; Russell Sage Institute of Pathology, New York, N. Y.; Johns Hopkins University, Baltimore, Md.; University of Rochester, Rochester, N. Y. A large animal calorimeter was built by the late Dr. Armsby at Pennsylvania State College, State College, Pa. The first calorimeter built in this country was erected in the laboratories of Wesleyan University, Middletown, Conn., by the late Professor Atwater. This work was transferred to Washington, D. C., during the lifetime of Professor Atwater and later was resumed by a colleague, F. G. Benedict, at the Nutrition Laboratory referred to above. See page 22.

oxygen consumed, we may evaluate the character of the metabolism, and by estimating the oxygen consumed and the CO_2 given off, the respiratory quotient is found. This indicates the number of Calories due to the burning of glucid, lipid, and of protid. Knowing the respiratory quotient, and the amount of one of the foods named above, by using the chart on page 624, it is possible to determine the number of Calories derived from the other foods, or the character of metabolism, whether chiefly glucid, protid, or lipid. If the subject is on a high glucid diet, his data will be found in the right-hand corner of the chart; if fat, in the left-hand corner. The

Fig. 184.—Method of administering the Douglas bag in collecting respired air for R/Q determinations. After a few minutes' breathing through the mouthpiece to the outer air, the nurse carefully closes the valve, permitting the patient to breathe into the bag.

figures are in percentages. The chart is arranged for the average respiratory quotient, namely, 0.83.

The Tissot[1] *Method with the Douglas Bag.*—The following apparatus is necessary:

Douglas bag (Fig. 184) or equivalent for collecting the respired air. The tube leading from the patient's mouth to the bag must be of large bore, about the size of the tubing on a vacuum cleaner. Unless a gas-mask is used, the mouthpiece must be provided with rubber flanges, which fit inside the cheeks to insure that no air escapes. An easily adjusted "flutter-valve" which can be operated without the patient's knowledge is provided, whether or not the mask is used.

[1] Tissot (French physician), J. de physiol. et de patholog. générale, vol. 6, p. 688, 1904.

A gas-meter, capable of recording the number of mls. of gas passed through it from the bag, in order to determine the total amount of gas being analyzed for CO_2 and O_2.

A gas-analysis apparatus (Haldane, Carpenter,[1] Henderson,[2] etc.), of which there are several different kinds on the market, the simplest of which being that of Henderson (Figs. 186, 187).

Procedure: The subject lies on his back with a good supply of fresh air available. The mouthpiece of the apparatus is applied, the valve being open to the outer air. The subject is instructed to inhale and exhale regularly several times before the valve is closed.

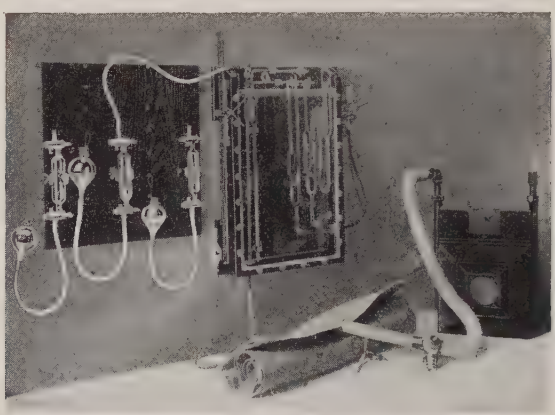


Fig. 185.—Newcomer modification of the metabolism apparatus of Haldane and others. Respired air is collected in the Douglas bag, the volume of air determined by the ordinary gas-meter and analyzed on the Haldane gas-analysis apparatus. Sampling tubes are seen suspended from the wall. See Newcomer, H. S. (Phipps Institute, Philadelphia), Jour. Biol. Chem., vol. 47, p. 489, 1921.

The latter is closed[3] at the end of an inspiration and the patient then inhales from the open air and exhales into the bag for from eight to ten minutes.[4] At the end of this time the valve is opened, thus permitting the patient to exhale into the open air and at the same time closing the bag. The mouthpiece is removed and the bag is then

[1] Carpenter, T. M. (Nutrition Laboratory, Boston, Mass.), Jour. Biol. Chem., vol. 55, p. xix (Proceedings), 1923.

[2] Henderson, Y. (Yale University, New Haven, Conn.), Jour. Biol. Chem., vol. 33, p. 31, 1918.

[3] An attendant closes the valve without the knowledge of the subject, in order to avoid subjective effects.

[4] In the author's experience, eight minutes is the average optimum time for the collection of respiratory air; beyond this time, the subject becomes restless and begins to feel aware of the unusual procedure.

attached to the gas-meter. Pass several hundred mls. of the respired air through the meter, then collect a sample of the gas for analysis in the Henderson apparatus. The total amount of gas in the bag is determined by continuing the passage of the gas through the meter. The sampler, working by a mercury level, is attached to the Henderson apparatus and the CO_2 and O_2 are determined by the

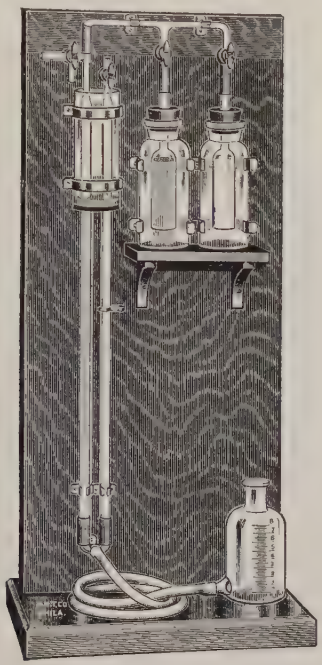


Fig. 186.—Henderson modification of the Orsat apparatus for analysis of respired air. Oxygen is determined by absorption from a small, known volume of the air, by means of phosphorus sticks in the left-hand absorption bottle and CO_2 by absorption in the right-hand bottle. For more critical work, see apparatus in Fig. 187.

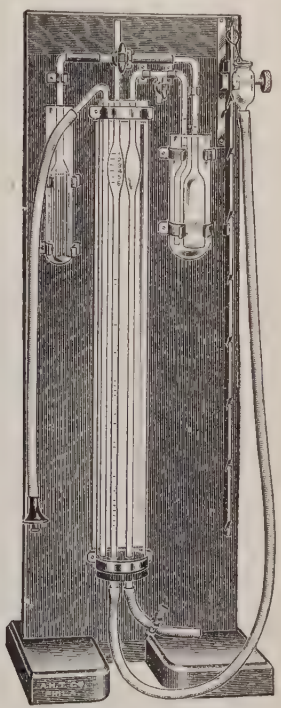


Fig. 187.—Improved form of gas-analysis apparatus; Henderson's modification of the Haldane instrument. Compare Fig. 186.

ordinary procedure of the gas-analysis apparatus; oxygen from a given volume of the sample air is absorbed by pyrogallol and CO_2 by moist soda lime, or other absorbent. The volume of the gas in the sampler is known and the total volume has been determined by the gas-meter. By simple calculation the gaseous exchange for the period of eight or ten minutes is found. It is necessary, for comparison, to make an analysis of the air of the room from which the Douglas bag was inflated at the beginning of the collection; or, if the air is

obtained directly from a free atmosphere, the following data may be used:

Oxygen concentration of air, 20.93 mls. per 100 mls. of air.
Carbon dioxid concentration, 0.3 ml. per 100 mls. of air.

Oxygen may be used in place of air.

Spirometer Method.—In this method the bag is replaced by a spirometer of large dimensions, from which the patient takes oxygen either in the form of pure oxygen or as air. He inhales and exhales into the spirometer. The rest of the procedure is the same as for the first method.

Method by Oxygen Utilization.—Principle: By this method the amount of oxygen utilized by the subject from a spirometer full of oxygen from an oxygen cylinder is determined for a given period of time. A circuit of the gas is maintained by means of a respiratory valve from the spirometer to the mouthpiece and thence through the soda lime absorber which may be either within the spirometer or outside. Finally, the current of gas is returned to the spirometer. The soda lime takes up both CO_2 and water-vapor from the circulating gas and, therefore, the contents of the spirometer remain as pure oxygen gas. An index, graduated in milliliters on the outside of the spirometer, enables one to read the volume of oxygen placed in the spirometer and also the content at the end of the run. The difference indicates the amount of oxygen utilized by the subject for the period of the determination, or a graphic record is obtained from the use of a kymograph (Fig. 188).

Procedure for the *Roth* Method: With the subject lying comfortably on his back, wheel up the calorimeter, and by means of the adjustable arm bring the mouthpiece directly over the subject's mouth. Adjust the mouthpiece with the flanges within the subject's cheeks. Have him inspire and expire for about two minutes, and then, at the end of an expiration, open the valve in order to permit him to breathe from the spirometer. Note the time. At each expiration note that the spirometer does not return to the mark at which it rested at the beginning of the previous inspiration; oxygen is being utilized and the CO_2 from the breath is being absorbed by the soda-lime. If the subject becomes restive, the run may be discontinued. Terminate the run on the even minute for ease of calculation. Read off the volume of O_2 utilized from the spirometer and the temperature from the thermometer. Enter these data in your note-book. Calculations will be made later (page 622). If the

kymograph is used, one revolution of the drum is made in fifteen minutes, or 33 mms. per minute.

The following description applies to the apparatus known as the Sanborn instrument, which is a modification of the Benedict[1]

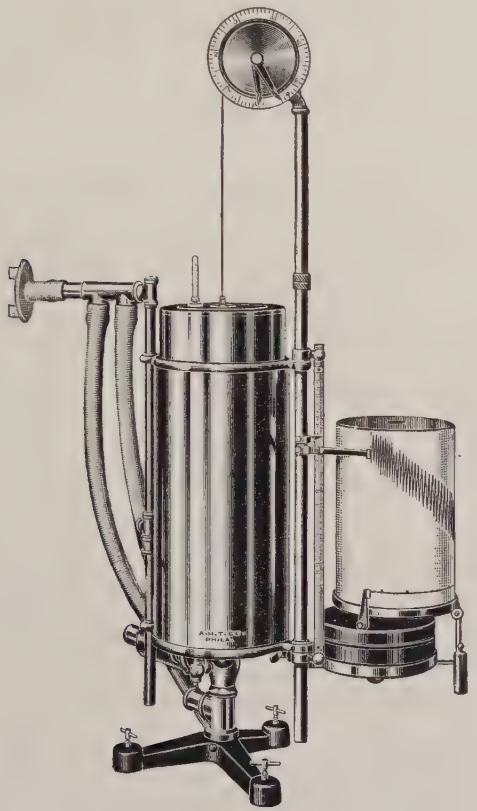


Fig. 188.—The Roth modification of the Benedict basal metabolism apparatus. This instrument avoids the use of an electric blower for circulating the gas through apparatus and also incorporates a means of graphically recording the findings on a kymograph (right). The mouthpiece is shown attached to the arm extending to the left. The spirometer is the large cylinder in the middle of the picture, with its bell floating in water.

apparatus; this was described in the Boston Medical and Surgical Journal, vol. 178, page 667, 1918.[2]

Procedure: Testing the apparatus: Open the valve leading to the mouthpiece and elevate the floating cylinder of the spirometer to

[1] F. G. Benedict (page 23).

[2] Two main changes have been made: (1) Removing the motor to the outside of the spirometer, and (2) enclosing the absorber within the spirometer. See Boston Med. and Surg. Jour., vol. 183, p. 449, 1920.

7000 mls., indicated on the graduated scale. Place two 100-g. weights on top of the cylinder, one on either side of the center, and close the valve. The cylinder should remain stationary. If it does not, submerge each part of the tubing and its connections under water to determine where the leak is; in the instrument provided with a circulating pump[1] there is frequently trouble of this sort. Having repaired the leak, empty the cylinder, and fill with oxygen through the small bibb-cock below the spirometer.

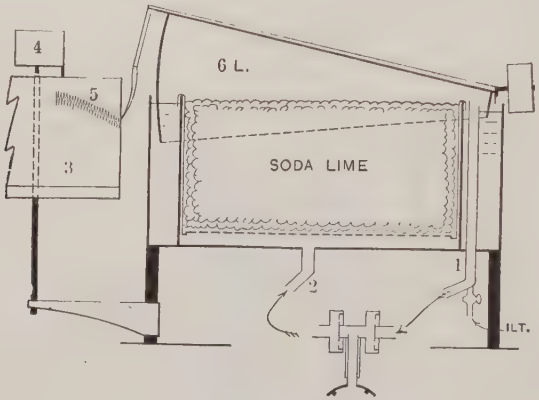


Fig. 189.—Krogh's calorimeter. The hinged compartment 6L. replaces the spirometer of the methods described on pages 612 and 613. A kymograph, 5 to 3, serves the same purpose as that in the Roth apparatus (page 613). The mouthpiece for the patient is connected with Tubes 1 and 2, below. The CO_2 is removed by means of the soda lime. Oxygen gas is admitted to the apparatus through 1*LT*. (From DuBois, "Basal Metabolism in Health and Disease," Lea & Febiger, Publishers.)

The remainder of the procedure is like that of the Roth apparatus (Fig. 188).

The Graphic Method.[2]—*Method of Krogh.*[3]—The apparatus is a rocking spirometer,[4] with an index attached to record the fall of the spirometer bell as oxygen is used by the subject. The pointer writes on a kymograph-paper. In the spirometer are placed 8 kgs. of moist

[1] The Roth apparatus first described is a modification of the Benedict form; in former, respiratory valves are substituted for the rotary blower. See Boston Med. and Surg. Jour., vol. 186, p. 457, 1922; vol. 186, p. 491; vol. 189, p. 551.

[2] The Roth method utilizes a chronokymograph, a rotary cylinder upon which a pen writes, thus indicating the excursions of the spirometer cylinder.

[3] A. Krogh (Copenhagen; Nobel-prizeman in physiology, 1923), Wiener klin. Wochenschr., volume for 1922, No. 13. See also Boston Med. and Surg. Jour., vol. 189, p. 313, 1923. The original description was in the Skandinavisches Arch. f. Physiol., vol. 27, p. 100, 1922.

[4] The principle of the spirometer is that of Gad; see figure in Brubaker, A. P., Text-book of Physiology, Philadelphia, P. Blakiston's Son & Co., 8th ed., 1925, p. 431.

soda lime for absorbing the CO_2, this amount being adequate for several hundreds of analyses. The gas is absorbed from the spirometer, passes to the subject, and thence is breathed back into the soda lime. The kymograph rotates at the uniform speed of 20 mms. per minute. Records are made in ink. At the beginning of the run 5 liters of pure oxygen are added to the spirometer through the tap attached to the pipe leading to the spirometer. The gas-mask or mouthpiece leading from the spirometer is fitted to the subject and the run is started. The first five minutes are disregarded, owing to the abnormally high oxygen absorption due to the rise of O_2 content in the spirometer. The run is made for about ten minutes. As before, the temperature, barometric pressure, and oxygen consumed are entered into the note-book. The last item is obtained from the paper, which is removed from the drum. The ten-minute period, indicated by vertical lines, is exactly 200 mms. long. A scale, calibrated in liters of oxygen, accompanies the apparatus. The scale is applied to the vertical line and the reading is taken. Then apply to the second vertical line, and the difference gives the amount of O_2 consumed during the run.

What Substances Produce Heat Energy?—This question was answered by Lavoisier,[1] who used a small ice-calorimeter[2] capable of holding an experimental animal. The amount of ice melted by the animal after a certain interval (ten hours) gave the data necessary for determining how many calories of heat pass from the animal within that time. By considering that carbon was burned and gave rise to that energy, Lavoisier was able to account for only 81 per cent. of the total amount, but by considering that hydrogen was burned, likewise, he arrived at 96 per cent. of the theoretical amount. We know now that heat coming from an organism is due to the burning of carbon to CO_2 and the oxidation of hydrogen to H_2O. If simply elementary carbon and hydrogen were burned, we could at once state the amount of heat arising from the burning of these elements, but our foods do not furnish just C and H_2; the carbon and hydrogen of our foods are in the form of compounds like glucids, lipids, and protids. Also, decomposition accompanies oxidation. Now all atomic oxidations are exothermic, that is, heat is given off whenever an atom unites with oxygen, thus:

$$C + O_2 = CO_2 + 96.7 \text{ Cals.}$$

58 Cals. with water as steam, or

$$H_2 + \tfrac{1}{2}O_2 = H_2O + 69 \text{ Cals. with water as liquid water,}$$

[1] Page 21. [2] See Mathews, page 283.

but reactions in which compounds are oxidized, decomposition of the reacting compounds frequently necessitates adding heat from without and this part of the reaction is endothermic. Then, when oxidation occurs, heat is given off, but it is necessary to subtract the heat which had to be supplied to decompose the substance prior to oxidation. Moreover, there is the specific dynamic action of the foods[1] discussed on page 565. Altogether, as a basis of indirect calorimetry it is necessary to determine by actual experiment the yield in calories of food substances. For the three typical foods:

	Calories.
1 g. glucid as starch yields	4.116[1]
1 g. lipid yields	9.353
1 g. protid yields	4.124[2]

[1] Slater, W. K. (University of Manchester, Manchester, England), Biochem. Jour., vol. 18, p. 621, 1924. 1 g. of pure glycogen from the French clam gives 3.836 Cals.

[2] About 5.5 when burned outside the body; the difference is due to the fact that the body does not completely oxidize the nitrogenous part of the molecule; see page 539.

By comparing these figures with the theoretical calculated from food calorimetric determinations, the "anomaly" can be found.

The **respiratory quotient** depends upon the character of the metabolism. By means of it the character of metabolism is determined. We shall discuss the R/Q for the three typical food materials:

(1) *The R/Q for Glucids.*—Glucose is taken as a basis for consideration:

$$C_6H_{12}O_6$$
Glucose.

or we may express it:

$$6C(H_2O)_6$$

It is evident that if glucose is burned, only 6 atoms of carbon will require oxygen from without, because intramolecular oxidation of the hydrogen by the oxygen contained in the molecule will take place. Six CO_2 molecules are formed on oxidation of the carbon, that is:

$$6C + 6O_2 = 6CO_2.$$

Therefore:

$$\frac{CO_2}{O_2} = 1.$$

The respiratory quotient for glucids is therefore unity.

Another simple method of calculation may be given: Taking starch:

$$C_6H_{10}O_5 + 6O_2 = 6CO_2 + 5H_2O.$$

[1] In thermochemistry any external effect upon a thermochemical equation is known as an anomaly, and in biochemistry the term "protein anomaly" is encountered, meaning that some factor other than stoichiochemistry is involved.

Since 1 molecular weight = 22.4 liters,[1]

$$6O_2 = 6 \times 22.4 \text{ liters, or } 134.5 \text{ liters.}$$

Likewise:

$$6CO_2 = 6 \times 22.4 \text{ liters, or } 134.5 \text{ liters.}$$

$$\frac{CO_2}{O_2} = \frac{134.5}{134.5} = 1, \text{ the R/Q for glucids.}$$

Whenever the amount of CO_2 excreted equals the volume of oxygen consumed for the same period, as determined by one of the methods given above, the subject is utilizing the sugars.

(2) *The R/Q for Lipids.*—Here we shall take, for illustration, a molecule of an unsaturated fat, since these substances seem to be those actually burned in the body:

$C_{57}H_{104}O_6$
Trioleïd.

Taking out as much intramolecular water as the oxygen in the molecule permits, we have H_2O, utilizing 12 hydrogens, leaving 92 hydrogens to be burned with oxygen furnished from the outside. Oxygen must be furnished to burn 57 carbons. Fifty-seven molecules of oxygen are required to burn the 57 atoms of carbon, and the 92 hydrogens, or 46 H_2 molecules, require half the number of O_2 molecules, or 23 O_2 molecules, making, in all, 80 oxygen molecules which have to be furnished to burn the molecule of fat. The R/Q, or $\frac{CO_2}{O_2} = \frac{57}{80} = 0.71$, the respiratory quotient for lipids.

Or, we may make the calculation according to the actual volumes of gases following the second method given under glucids: Taking the compound,

$$C_{51}H_{98}O_6 + 72.5\ O_2 = 51\ CO_2 + 49H_2O$$

$$R/Q = \frac{CO_2}{O_2} = \frac{51.0 \times 22.4}{72.5 \times 22.4} = \frac{1142.5}{1624.0} = 0.70 + \text{R/Q lipids.}$$

(3) *The R/Q for Protids.*—The respiratory quotient for protids is intermediate between that of glucids and the lipids. The burning of protid is virtually the oxidation of the amino-acids composing it. Some of the amino-acids, like glycin, have the same R/Q as glucids:

CH_2NH_2COOH
Glycin.

Omitting nitrogen, since glycin is oxidized after deaminization:[2]

$C_2H_4O_2$, or $2C\ (H_2O)_2$.
Oxidizable glycin.

[1] A gram-molecule of any substance in gaseous form occupies the volume of 22.4 liters.

[2] Page 540.

This gives $\frac{CO_2}{O_2} = 1$, R/Q for glycin.

Other amino-acids, like alanin, have a lower R/Q:

$CH_3CH.NH_2COOH = C_3H_6O_2$, or $3C(H_2O)^3$ giving $\frac{3.0}{3.5} = 0.85$, R/Q for alanin.

Taking the nineteen or twenty amino-acids and averaging their respective quotients in this manner we find the R/Q for protids 0.82.

Calories from Urinary Nitrogen.—The calories derived from protids may be determined by collecting the urine, estimating the total nitrogen, and then multiplying the number of grams of nitrogen by the caloric value of 1 g. of urinary nitrogen, 26.51 Cals. One hundred grams of protid when eaten will produce 431.63 Calories,[1] a determination made by direct calorimetry. There are 16.28 gs. of nitrogen in 100 gs. of the average protid; hence, 1 gram of urinary nitrogen gives $\frac{431.63}{16.28} = 26.51$ Cals.

Non-protid Calories.—Subtracting the protid Calories, determined above, from the total number of Calories estimated by any of the calorimetric methods gives the non-protid Calories (sum of Calories from glucids and from lipids). This quantity varies with the R/Q, as shown by the following table from Lusk after Loewy:

1 g. glucid.....	R/Q 1.00	5.047 Cals. per liter O_2
1 g. lipid.....	R/Q 0.70	4.686 Cals. per liter O_2

We are now in position to understand why the respiratory quotient varies with the different kinds of food: The oxygen present in the molecule in proportion to the carbon and hydrogen determines the respiratory quotient. Consequently, the amount of oxygen utilized by the individual is an index of the kind of metabolism occurring in the individual. In clinical diagnosis advantage is taken of this fact.

BASAL METABOLISM

By the term "basal metabolism" is meant the oxygen utilized by a subject at rest, on a maintenance diet and under normal conditions.[2] In abnormal states, the B. M. R.[3] is raised or lowered over the expected normal. The expected normal is estimated in terms of body characteristics. An increase or decrease in B. M. R. of 15 per cent. or more from the expected normal is considered pathognomonic.

[1] This amount of protid, when burned in a food calorimeter, yields 563 Cals., but in the body 131.46 Cals. are lost through the feces and urine.

[2] Krogh (see page 614) prefers "standard metabolism;" see Krogh, (page 634).

[3] Basal metabolic rate, measured by the oxygen utilization per hour per standard reference, *i. e.*, square meter of body surface.

The Determination of Basal Metabolism.— Basal metabolism may be determined by any of the methods outlined above, direct or indirect. In either case, it is necessary to have a standard normal for reference. *The standard normal is expressed in terms of unit body surface, but age, sex, and other conditions are factors.* The actively metabolic mass determines the Calories. The metabolism of a mouse is actually much less than that of an elephant, but proportionately, comparing the body surfaces, the two are quite similar. Estimated in terms of square meters of body surface, or fractions thereof, the heat production of "warm blooded"[1] animals is similar.

TABLE (AFTER LUSK FROM VOIT) SHOWING THE HEAT PRODUCTION IN DIFFERENT ANIMALS IN TERMS OF SQUARE METERS OF BODY SURFACE

	Kilos.	Calories.
Mouse, weighing	0.018	1188
Chicken	2.000	947
Dog (large)	51.500	1039
Pig	128.000	1078
Infant on breast	5.000	1221
Human dwarf	6.600	1231
Man, adult	64.300	1042
Dog (fasting)	12.000 (estim.)	1112
Man, adult (fasting)	50.000 (estim.)	1134

The metabolism of different animals estimated in Calories per square meters of body surface per day averages about 1100 Calories, or 45.85 Cals. per hour. The average heat production per square meter of body surface per hour is 40 Calories, since there are times in the twenty-four hours when the metabolism is much above B. M. R. In man this is true for the adult. In the young, there is an increase from birth to five years which is somewhat compensated for by a fall appearing at five years and progressing until death.

Measurement of Body Surface.—Obviously, the measurement of the surface of such an irregular body as that of an animal is not easily accomplished. The method proposed by French investigators was improved by DuBois,[2] who dressed his subjects in union suits and then pasted over this underwear strips of paper which did not stretch nor contract; then the suits were cut up and laid out flat, photographed, and the photographic reproductions weighed and the weights compared with that of photographic paper representing known area. By this means, Du Bois found a formula whereby the

[1] Animals lacking the thermostat regulator, the so-called "cold-blooded" animals, or poikilothermal forms (varying temperature), behave differently. See Murlin, p. 521.

[2] DuBois, E. F. (Medical Director, Russell Sage Institute of Pathology, New York, N. Y.), Arch. Int. Med., vol. 15, p. 868, 1915.

height and weight of the subject when modified by certain constants, could be expressed in terms of body surface, the formula being:

$$A = W^{0.425} \times H^{0.725} \times C,$$

where

A is the area (sq. cm.).
W is the weight in kilos.
H is the height in centimeters.
C is a constant, numerical value, 71.84.

By means of this formula, knowing the height and weight of the subject, the surface area may be calculated with an error of about ± 1

Fig. 190.—Method of estimating surface area by direct method. A cretin, Benny L., and his surface mold. (DuBois.)

per cent. A closer approximation, however, may be made by using the Harris-Benedict equation, which differentiates men and women:

For men: $66.4730 + (13.7516 \times \text{weight}) + (5.0033 \times \text{height}) - (6.755 \times \text{age})$.
For women: $655.0955 + (9.5634 \times \text{weight}) + (1.8496 \times \text{height}) - (4.6756 \times \text{age})$.

This method gives results about 4 per cent. lower than the DuBois' results. It is best used with an average subject.

Relation of Oxygen Consumption to Basal Metabolism.[1]—The amount of oxygen utilized by the respiration of the subject[2] is deter-

[1] See Boothby and Sandiford, and also DuBois.

[2] Page 605.

mined by one of the methods given above. The problem remains to convert it into Basal Metabolic Rate. Let us assume that the figure obtained is for ten minutes. The utilization rate per minute is 0.1 of the whole amount of O_2, used from the spirometer, or equivalent. Again, since such determinations may be made at Atlantic City (barometric average, 760 mms. Hg), at Denver (bar. average, 640 mms.), or elsewhere, it is essential to the comparison of oxygen utilization that the volume of O_2 be reduced to standard conditions, 760 mms.

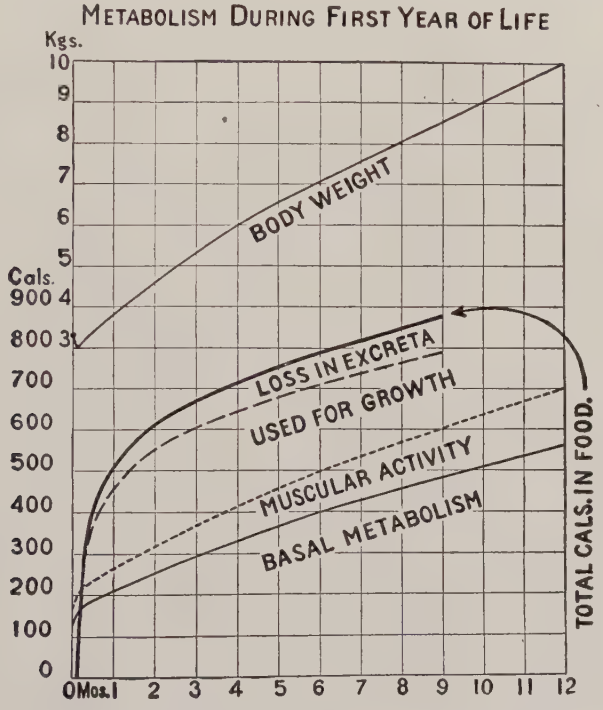


Fig. 191.—Chart showing metabolism during first year of life. (Talbot, Amer. Jour. Dis. of Child., vol. 17, Chart 2, p. 231, 1919.)

Furthermore, since a gas volume varies with the temperature, this correction must be made. The two quantities, pressure and temperature, may be reduced to standard conditions simultaneously by the following equation:

$$\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2},$$

where P_1V_1 are the standard pressure and volume sought at standard temperature, T_1, expressed on the absolute scale (zero being $-273°$ C.); P_2V_2 represent the pressure by barometer when the determina-

tion is made and the volume observed on the scale of the spirometer; and T_2, the temperature on the thermometer attached to the spirometer, expressed in degrees Centigrade + 273. By solving for V_1, the volume of oxygen absorbed is expressed in standard volume.[1] Considering the subject to be upon an average maintenance diet, where the respiratory quotient is 0.84, 1 liter of oxygen used is equivalent to the expenditure of 4.85 Cals.[2] of energy. For one hour this amounts to sixty times as much. Then:

Calories per hour = Number of mls. O_2 used $\times$ 60.

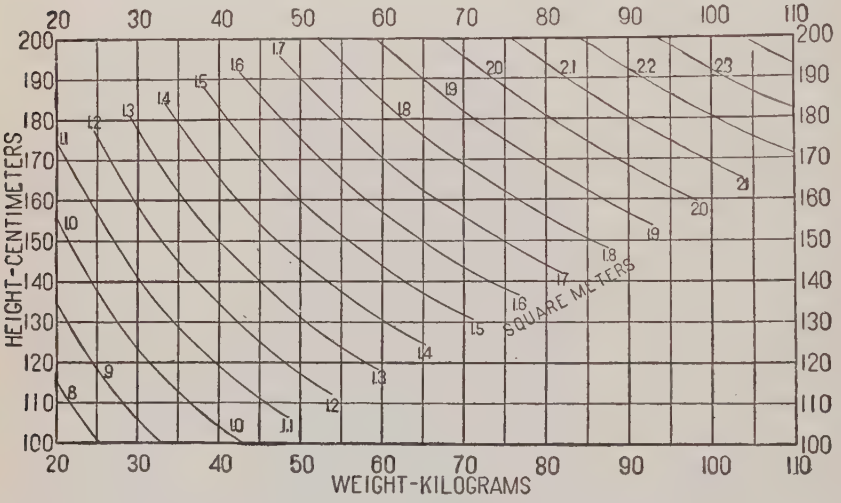


Fig. 192.—DuBois chart for determining surface area of man in square meters from weight in kilograms (Wt.) and height in centimeters (Ht.) according to the formula: Area (Sq. cm.) = $Wt.^{0.425} \times Ht.^{0.725} \times 71.84$. (DuBois.)

For the comparison of different individuals, some large, some small, it is necessary to express this quantity in square meters of body surface. This is done by solving the equation on page 620 (top) and then we have the necessary data for determining the basal metabolic rate (B. M. R.):

$$\frac{\text{Calories found as above}}{\text{Square meters body surface}} = \text{B. M. R. (calories per square meter per hour).}$$

B. M. R. is recorded as percentage above or below a standard expected from the height-weight and other data for the subject. This is determined by the following:

[1] Other corrections for nitrogen of the air, respired air, etc., are discussed on page 84 of Boothby.

[2] This is exclusive of the "non-protid" R/Q, which amounts to 0.3, and hence is unimportant.

(1) If the expected rate is more than the rate found:

$$\text{Per cent. B. M. R.} = \frac{\text{Expected rate} - \text{rate found}}{\text{Expected rate}} \text{ gives} - \text{per cent.}$$

(2) If the expected rate is less than the rate found:

$$\text{B. M. R.} = \frac{\text{Rate found} - \text{expected rate}}{\text{Expected rate}} \text{ gives} + \text{per cent.}$$

When the protid is not involved in this determination it is assumed that there may be variation either above or below the expected normal of 15 per cent., without being pathognomonic. If the subject is a man aged forty-five, the normal standard B. M. R. is about 38.5 Cals. per sq. cm. per hour, and if his B. M. R. proves to be 43.6 Cals., his percentage B. M. R. is $\frac{43.6 - 38.5}{38.5} = +0.13$, or 13 per cent.

Normal Standards.—The chart of DuBois on page 622, Fig. 192, gives at a glance the surface corresponding to the weight of the subject in kilograms and the height in centimeters. Thus, if one enter

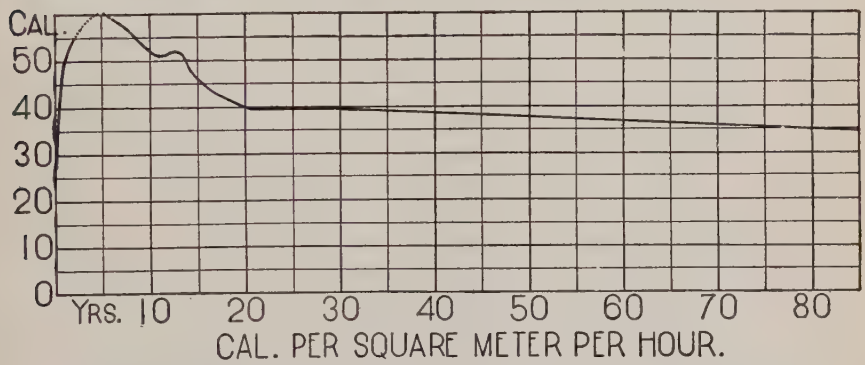


Fig. 193.—Basal metabolism, as measured in Calories, produced per square meter of body surface per hour from birth until the age of eighty-five years in human males. Between maturity and the eighty-fifth year there is a gradual fall in the intensity of metabolism of 13 per cent. (DuBois.)

the table with the data of a male adult 177.5 cms. in height and 63.5 kilos in weight, his body surface is 1.75 sq. m. A normal person will give for this extent of surface about 10 Cals. per hour, which, reduced to 1 sq. m. per hour, gives 40 Cals. This figure is the same as was found from the total Calories in twenty-four hours estimated by oxygen consumption,[1] but in that case we specified an "adult man." The expected normal varies with age as well as with surface; hence, three factors must enter the determination of the expected normal—Height, weight, and age. Normal standards have been worked out

[1] Page 620.

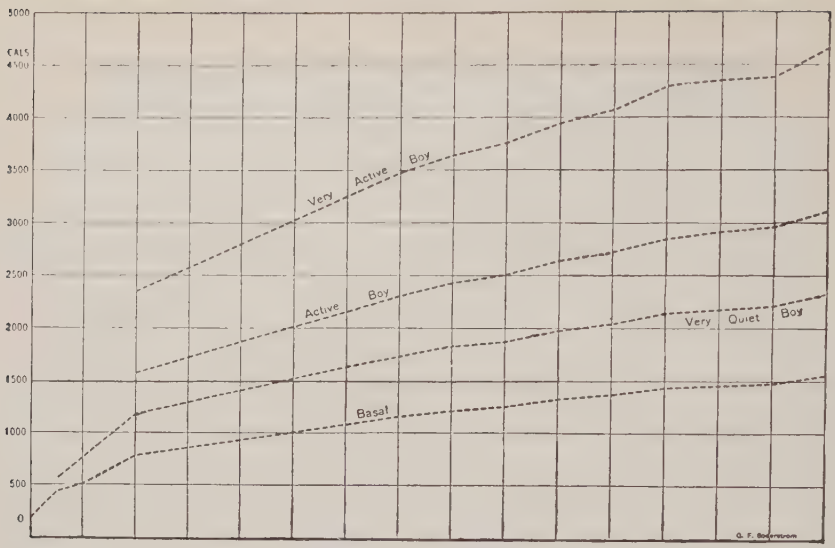


Fig. 194.—Comparative estimates of physiological needs for food of growing boys and girls. Ordinates, Cals., abscissæ, years. (Lusk, Jour. of Home Econ., Baltimore, vol. x, p. 281, 1919.)

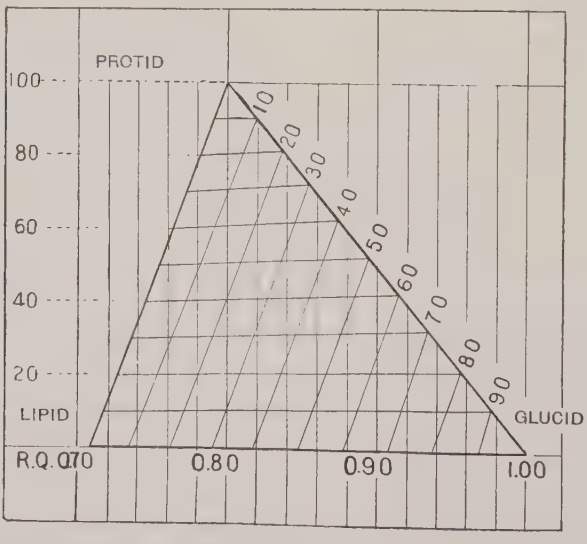
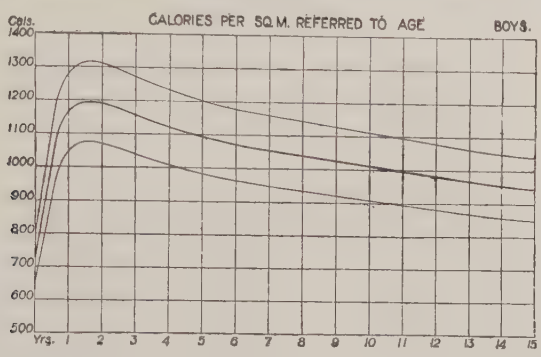


Fig. 195.—Du Bois chart for obtaining percentage of Calories from different classes of food substances, when the Respiratory Quotient and the number of Calories derived from protid food are known. Example: R/Q = 0.82; Cals. from protid, 18 per cent. Then: Percentage Cals. from glucids = 35 per cent.; from fat, 53 per cent. (From DuBois, Basal Metabolism in Health and Disease. Lea & Febiger, Publishers.) This form of graph was introduced by G. C. Stokes.[1]

[1] Proc. Roy. Soc., vol. 49, p. 174, 1891.

for various combinations by various investigators,[1] notably Benedict and his colleagues, and the tables are reproduced in the accompanying pages;[2] there are also tables by DuBois, which were published earlier.[3]

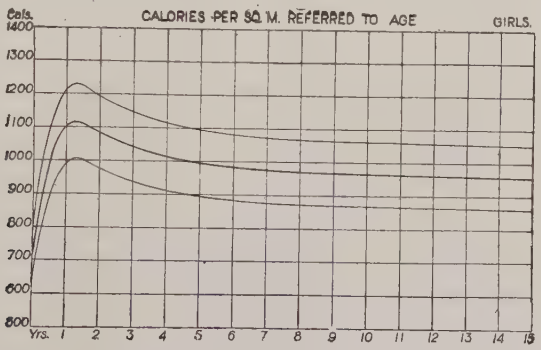
Use of the Tables. In order to predict the expected Calories per hour for adult males, enter Table A with the weight expressed in



BOYS' SURFACE AREA

High, medium, and low metabolism, boys. Per surface area. (After Talbot.)

kilos and find the corresponding Calories. Then enter Table B with the height expressed in centimeters and find the corresponding Calories. Add Calories from A to those from B. For adult females, use Tables A and C in a similar manner. For boys, use Table D.



GIRLS' SURFACE AREA

High, medium, and average metabolism, girls. Per surface area. (After Talbot.)

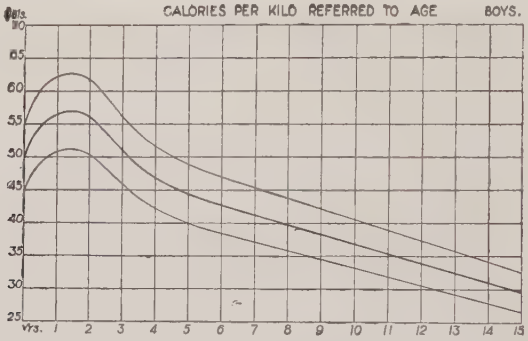
For girls, use Table E or Table F. Dreyer's table (G) gives the data for adult males; the same table may be used for small females if 10 per cent. be subtracted from the corresponding male Calories.

[1] See Lancet (British medical publication), vol. 199, p. 289, 1920, article by Dreyer.

[2] Appendix. See Carnegie Institution Publication Number 279. Washington Carnegie Institution of Washington, 1919. Also Number 303, 1921. For data concerning girls see Boston Med. Surg. Jour., vol. 188, p. 127, 1923. (See Carpenter.)

[3] See Du Bois.

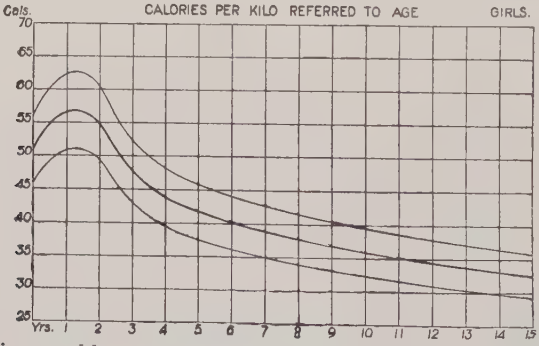
Normal Variations in Basal Metabolic Rate.— As stated (p. 623), the B. M. R. may vary about 15 per cent. on either side of the normal standard without indicating a pathological state. Attention has already been directed to the variation in sex and in age. As a rule, the female B. M. R. runs 7 per cent. less than that of the male. The



BOYS' WEIGHT

High, medium, and low metabolism, boys. Per weight. (After Talbot.)

variation in age is given in the graphs on pages 625 and 626. From birth to about three to five years the metabolism increases, then falls until old age. During a day's period at different ages the variations are characteristic. For the male and female, alike, the greatest daily variation occurs between forty-two and fifty-two years. In the



GIRLS' WEIGHT

High, medium, and low metabolism, girls. Per weight. (After Talbot.)

female this is a period of profound bodily change, involving structure and function. The variation from basal metabolism during the period of growth is shown in the graphs on pages 625 and 626. The actual energy requirements over basal rate are shown in Fig. 194 for boys of different temperament, and it will be noted that the energy requirement of a very active boy of nine is so increased that his B. M. R. equals that of an adult male (3700 Cals.).

In fasting, the B. M. R. is strikingly constant, as the following table of the metabolism of a professional faster shows (after Lusk[1]):

METABOLISM OF CETTI IN STARVATION

Fasting days.	Protid.	Fat.	Calories from protid.	Calories from fat.	Calories, total.	Calories per kilo.
1 to 4.....	85.88	136.72	329.8	1288.2	1618	29.00
5 to 6.....	69.58	131.30	267.3	1237.4	1504	28.38
7 to 8.....	66.30	149.35	254.7	1407.3	1662	31.74
9 to 10.....	67.96	132.38	261.1	1247.4	1508	29.26

Not only is this statement true for man, but for animals in general. The amount of protid in the food determines the level of metabolism as determined in Calories. Thus, in an experiment of Rubner, quoted by Lusk, in which a dog, previously on low protid diet, was fed much meat for several days, the Calories increased and when the protid was withdrawn, the metabolism continued on the elevated plane:

TABLE SHOWING THE INCREASE IN METABOLISM ON ADDITION OF PROTID TO THE DIET[1]

Calories in meat ingested.	N retained by body.	Carbon retained by body.	Total calories of metabolism.
0	−1.31	....	310.61
0	−1.52	....	278.00
481.5.....	3.95	2.97	311.43
481.5.....	2.80	3.70	333.82
481.5.....	2.30	1.61	368.41
481.5.....	2.20	2.53	361.70
481.5.....	0.92	4.45	375.47
481.5.....	0.20	4.31	395.77
0	−3.70	....	357.20
0	−2.64	....	310.29

[1] Note that nitrogenous equilibrium is reached on the first day of protid feeding, and that, as the carbon shows, there is a storage of organic substances.

Fat, when given alone, **exerts a specific dynamic action,** as we have said on page 566, the extra metabolism being solely due to fat. If fat be given with protid, however, there is no increase in metabolism, even if there is such an excess of fat over body requirements as to lead to fat deposition.[2] This fact is borne out when the metabolism of a fat subject is compared with that of a lean one, for, although the total metabolism is greater in the case of the fat individual, yet when estimated as Calories per hour per unit of body surface, the metabolism of the two subjects is similar.

[1] Lusk, page 22.

[2] In this case the specific dynamic action of fat does not become evident.

The **starches** and **sugars,** as we have shown, when fed in "plethora" amounts, **exert a specific dynamic action,** increasing metabolism. This action is greatest when glucids are not immediately utilized; an excellent illustration is fructose, which is less readily retained in the glycogen-forming organs, and hence passes to the tissues, where it protects the fat and leads to increased production of energy. One hundred grams of fructose cause 10 per cent. more energy development than an equal amount of glucose, and the respiratory quotient shows that the fructose is converted into fat. Glucose causes an increase in Calories of 9 per cent., due to its own combustion, as indicated by the Respiratory Quotient:

Minutes.		Percentage increase over B. M. R.
100 gs. glucose by mouth, R/Q after		
60	0.91	3
120	0.89	11
180	0.88	9
240	0.90	6
200 gs. of glucose by mouth, R/Q after		
60		
120	0.92	24
180	0.93	16
240	0.95	8
300	1.00	7

Fructose, on the other hand, causes an increase in Calories because it is more readily converted into fat, and the fat burns, giving larger amounts of heat. Glucids, when fed with protids, cause increased development of Calories. Thus, 50 gs. of glucose plus 20 gs. of the amino-acid glycin, produce the same amount of heat as 66 gs. of glucose fed alone, involving an increase in Calories of 53 per cent., which is more than the 66 gs. of glucose alone produce. Glucids fed with lipids cause increased heat production, but only to the extent that each of the substances would do if fed separately. The R/Q being less than unity (0.93), indicates that fat and glucose are burned at the same time. It is immaterial whether fat or sugar is the diet as far as metabolism is concerned.[1]

Insulin causes an increase in B. M. R. only[2] when it induces hypoglycemic convulsions, *i. e.*, blood-sugar 0.069 g. per 100 mls. blood, in the case of Boothby and Wilder. Hitherto, reports have been made to the effect that insulin augmented the B. M. R., but Boothby finds that CO_2 elimination is increased on administering insulin,

[1] See, however, page 572 regarding fatigue.

[2] Boothby, W. M., and Weiss, R., Jour. Biol. Chem., vol. 63, p. i (Proceedings), 1925.

because it reduces the CO_2-combining of the blood; the result is that the numerator of the ratio $\frac{CO_2}{O_2}$ is increased, which brings the quotient nearer unity. Hence, it is impossible to conclude that insulin causes an increased respiratory quotient, or augmented B. M. R. when there is an increased elimination of CO_2.

The **effect of muscle work** on the production of heat is to incite metabolism, and this results in: (1) Greater mechanical work, and (2) greater heat production. It is probable that the second factor aids the first and perhaps causes it; the extra heat produced is at the expense of the fat, the extra mechanical energy at the expense of the glucose. Mechanical work is more efficient on a high diet of glucose than on an increased protid diet. The protid when fed alone to a man at rest causes a similar increase in Calories, due to its Specific Dynamic Action, which is analogous to the increased metabolism of fat and sugar occurring in muscle work. Thus:

A man at rest and fasting produces 1976 Cals.

A man at rest fed 600 gs. sugar produces 2023 Cals., increase 2.4 per cent. over B. M. R.

A man working fed 600 gs. sugar produces 2868 Cals., increase 45.2 per cent. over B. M. R.

A man at rest fed meat in large amount produces 2515 Cals., increase 27.2 per cent. over B. M. R.

A man working fed meat in large amount produces 3370 Cals., increase 70.5 per cent. over B. M. R.

It is evident that the increase in Calories attributable to work is the same for sugar as it is for meat, and that the Specific Dynamic Action of the meat alone causes an increase in Calories nearly as great as that due to muscle work (2868 Cals., compared with 2515 Cals.).

The **effect of outside temperature and of clothing on the heat production** during exercise is negligible. The only effect attributable to these factors is the interference with heat loss, which is evident in fat subjects in whom fat inhibits the loss of heat from the skin; consequently, when outside temperature rises to nearly blood temperature (37° C.), metabolism rises and heat is not lost rapidly enough.

Effort or **strain** does not affect the heat production of the body nor do nervous effects attributable to fatigue cause any augmentation. However, after prolonged exercise, the metabolism is on a higher plane, even during rest, but the more accustomed a subject is to muscle effort, the sooner will his metabolism descend to the normal; this is a test applied in the evaluation of men for the army.

Beverages, like tea, coffee and cocoa, do not cause an increase in metabolism unless fortified with food.

Size of body is a factor in the efficiency of muscle exercise, a lean man of small stature requiring a greater expenditure of energy to effect the same amount of work than a lean man of large size. A normal man, weighing 86.5 kilos, walking horizontally expends 0.219 kilogrammeters of energy per kilo of body weight, whereas a man of 65.8 kilos body weight requires an expenditure of 0.230 kilogrammeters for every kilo of body weight per meter traveled.

Speed also influences the degree of energy expended; a normal man moving at an ordinary rate expends more energy proportionately than when walking fast, and the same is true for running. The case just mentioned, where the weight is 86.5 kilos, when moving at an ordinary rate expends 0.219 kilogrammeters per kilo of body weight per meter, but if he move very slowly he expends 0.233 kilogrammeters per meter. A man walking at about 5 miles per hour expends an average of 0.932 small calories per kilo of body weight per meter. Running at 5.3 miles per hour, he expends 0.816 small calories per kilo of body weight per meter.

The **position of the body** is a factor in energy production and should be given careful attention both by the physician in the case of the sick and by the basal metabolism technician. Metabolism determined with the patient in the dorsal position[1] is 0.05 Calorie per minute lower than when he is in a sitting position. If the patient stands while the determination is made, there will be further increase of 0.11 Calorie per minute. However, if the patient leans upon a support, the figures will be the same as those for the sitting posture. The B. M. R. of a soldier at "attention" is 1.99 Calorie per minute higher than his rate would be if taken in the dorsal position.

Variations in Metabolism Due to Disease.—*Goiter.*—A person with hyperthyroidism expends much more energy in the performance of a given task than a normal person:[2]

	Calories per kgm.
A person normal with respect to thyroid expends	1.20
A person having exophthalmic goiter expended	2.24
A person having adenoma with hyperthyroidism	2.86
A person having myxedema (hypothyroidism)	1.36

The average B. M. R. in myxedema is −28 per cent.; that in exophthalmic goiter, +52 per cent.; in adenoma with hyperthyroidism, +47 per cent. The effect of thyroidectomy may be determined by comparing the expenditure of energy in walking in a horizontal

[1] Lying on his back.

[2] See page 566 concerning the relation between thyroid and S. D. A.

plane before and after operation. Such a determination gave 2.16 small calories per kilogrammeter before and 1.77 to 1.35 after two weeks following an operation. In hypothyroidism the deviation from the normal B. M. R. is usually more than 15 per cent., and may reach −30 per cent. This permits a laboratory diagnosis based on basal metabolism determination, which is useful in clinical diagnosis of goiter cases. When thyroxin is used it gives 1.008 small calories per milligram of thyroxin, but the effect is delayed and not immediate as in the administration of epinephrin. Boothby and Sandiford find that dried thyroid tissue may supplement thyroxin; one gram of desiccated thyroid orally administered tissue has the same power to increase heat production as 2.5 milligrams of thyroxin given intravenously, according to the following equation:

Increased heat production in Cals. = 800 $\sqrt[3]{T}$, T being the daily dose of thyroxin necessary to produce the increased heat production.

Diabetes Mellitus.—About 70 per cent. of patients with diabetes mellitus show no abnormality in the B. M. R., but on a high protid diet, or one high in heat producing capacity, metabolism is increased. The increase in metabolism reported by some investigators has been shown by Joslin[1] to be due to the high protid and fat diets. The following figures from Boothby and Sandiford show the distribution of Calories in 61 cases of diabetes mellitus:

	Cases.
Basal M. R. below −20 per cent	5
From −20 to −16	3
From −15 to −11	5
From −10 to +10 (normal)	69
From +11 to +15	13
From +16 to +20	2
From +20 to higher	3

The number of cases in which there is a normal B. M. R. (−15 to +15), 87 per cent. The effect of insulin on B. M. R. is indirect, causing an increase in utilizable glucose and a stimulation of the conversion of fat into utilizable glucose; more heat is produced. Insulin itself does not cause acceleration of metabolism.[2]

Leukemia.—The B. M. R. is increased in leukemia, which one would scarcely expect, owing to the inactivity caused by muscular

[1] Joslin, E. P., American physician, Harvard University. See Treatment of Diabetes, Philadelphia, Lea & Febiger, 1923.

[2] In phlorhizinized dogs Ringer (Jour. Biol. Chem., vol. 58, p. 483, 1923) reported that insulin actually caused an increased oxidation of glucose, as witnessed by the increase in R/Q; see, however, page 628.

asthenia. The explanation that has been given by von Graefe[1] that the increased metabolism in leukemia is due to increased production of new cells is problematic, for, as we shall see, in pregnancy there is but little increase in basal metabolic rate, although production of new cells in the fetus[2] is prolific. The explanation probably lies in the plethora of amino-acids of the blood-stream due to autolysis of leukocytes, or it may lie in the presence of some specific substance stimulating cells to greater metabolism.

Pregnancy.—Although there is an abundant formation of new tissue during pregnancy, the metabolism is increased but slightly. The metabolism of a pregnant woman during the ninth month is higher than that during sexual rest, but the increase is due to the fact that two organisms are contributing, mother and child. The metabolism of the pregnant mother is about equivalent to that of the mother a week after delivery plus that of the child on the same date. Thus, it is evident that the effect of pregnancy on metabolism is practically negligible. During menstruation the B. M. R. is usually normal.

Anemia.—Blood-letting, following by transfusion to restore the volume of lost blood, causes no abnormal increase in B. M. R. In pathological anemias there is an increase in metabolism in about one-third of the cases, as shown by the following figures (Boothby and Sandiford):

Out of a total of 36 cases:	Per cent.
Below −20	3
From −20 to −16	0
From −15 to −11	3
From −10 to +10 (normal)	53
From +11 to +15	8
From +16 to +20	19
From +20 and higher	14

Number of cases considered normal (−15 to +15) 64 per cent. Number showing increased metabolism, 36 per cent. As the hemoglobin falls, metabolism rises.

Nephritis with Edema.—Since edema increases the body surface but not active metabolic mass, water being inactive, the metabolic rate is lower than the expected; oxygen is used to a less extent than the increased body surface calls for. All forms of edema affect the metabolism in a similar manner.

[1] von Graefe, A., German ophthalmologist, thyroid investigator, author of the "von Graefe sign" used in clinical diagnosis with respect to goiter.

[2] The metabolism of the fetus is a part of the general metabolism of mother and fetus.

Obesity was considered from the earlier investigations of Means[1] to vary in a manner similar to that of the edematous state, that is, involving inert material incapable of producing calories, but if the B. M. R. is calculated on a more correct formula (that of DuBois) the metabolism of the obese is found to be normal. When the outside temperature is lower than blood temperature (37° C.), metabolism is lower because of the factor just mentioned, *i. e.*, inertness. As stated before, interference with the loss of heat tends to cause a rise in body temperature which, in turn, affects metabolism and causes increased oxygen consumption.

From the foregoing discussion it is evident that the clinical value of metabolism determinations chiefly centers around the thyroid.

SUMMARY

(1) Energy requirements and the dissipation of energy in the body are expressed as heat units, or Calories. Isodynamic foods are those having the same caloric value in nutrition. Other factors than heat value, however, enter into the question of the value of foods in nutrition.

(2) A food calorimeter gives the value of foods when utilized in the body.

(3) This figure, however, is modified by special properties of the different foods, known as their Specific Dynamic Action and their ability to be completely oxidized in the body.

(4) In order to determine the actual value of any food, the energy it gives when burned in the body must be determined.

(5) The two chief means for the estimation of energy expended by the body are: (1) Physical or direct determination, and (2) chemical or indirect; the (2) is determined either by: (*a*) The amount of energy is estimated from the oxygen used and the products of oxidation appearing after oxidation; or (*b*) the oxygen utilized, alone, is determined.

(6) Inasmuch as the amount of oxygen necessary to burn the different kinds of food in the body differs according to the food, the relation between the products of oxidation and the oxygen consumed varies with the kind of food.

(7) The quotient of the ratio $\frac{\text{carbon dioxid exhaled}}{\text{oxygen consumed}}$ is known as the Respiratory Quotient, abbreviated R/Q. For fats the R/Q is 0.70, for glucids 1.0, and for protids 0.82.

[1] Means, J. H. (Mass. Gen. Hosp., Boston), Journ. Biol. Chem., vol. 21, page 263, 1915.

(8) Clinically, the oxygen absorbed is expressed in unit of time per unit of surface-extent, sex, and age. This is compared with the normal standards and the per cent. above or below indicates the variation from normal metabolism. A ± 15 per cent. variation is considered within the normal range.

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CHAPTER XIV

METABOLIC ADJUVANTS

"Medicine owes no thanks to him who, without proof, would formulate her theories."—*Elliott*, quoted by Hoskins.

Correlation in the Body.—The human body, being an organism,[1] all its parts are correlated. There are two great systems which accomplish this correlation: (1) The nervous system, and (2) the chemical system. The chemical system has two parts: (*a*) The endocrine system,[2] which has to do with the substances produced by organs of the body, and (*b*) the vitamin system, which is concerned with substances produced by plants. Both systems control substances which are essential to the proper performance of bodily functions. At the present time the function of the vitamin system is not clear, but it is certain that the same fundamental principle is involved in both endocrine and vitamin, namely, the production of substances in one tissue which exert a profound effect upon another tissue. Vitamins have already been referred to,[3] but they will be reviewed here in the light of their practical interest in medicine. It is significant that our knowledge of the vitamins arose through the study of deficiency diseases known as avitaminoses. It must be borne in mind throughout the following discussion that our knowledge of the endocrine and vitamin systems is still too incomplete to warrant formulating a complete theory of their relations to the human body.

It is possible that vitamins are necessary for animals for the same reason that certain substances like iron and manganese are to plants, namely, to stimulate certain reactions which, owing to the specialization, may become reduced or suppressed. An example of a metabolic

[1] Greek *organon*, an instrument. An organ is an instrument designed for a given purpose, and the body, or "organism," is an integral unit composed of many organs.

[2] Greek *endon*, within, and *krino*, to separate; that is, a system separating certain bstances commonly called internal secretions. Another term synonymous with sudocrine is cryptorhetic (Greek *krypto*, hidden, and *reo*, to flow). The substances are ennthesized by the body from precursors made by plants.

sy [3] Page 551.

adjuvant in plants is glucokinkin, the insulin-like substance found by Collip in many diverse kinds of plants. Plants which contain chlorophyl are able to manufacture their own food substances from inorganic sources. Animals do not contain chlorophyl and the utilization of their foods require green-plant adjuvants. Our recent knowledge of vitamin-like reagents, such as radiated foods, suggests that since the animal has largely lost its power to respond to the action of sunlight, so-called vitamins, or vitamin-carriers, like cod-liver oil must furnish the radiations, or special chemical configurations for human functions. It will be recalled that it is possible to replace the antirachitic vitamin by direct radiation of the human body, in which case we have the condition realized in plants. Our artificial habits of living, either out of the sunlight in houses or in using glass opaque to the effective rays of the sun, lead to "avitaminoses," which are counteracted only by supplying the deficit in some way.

AVITAMINOSES

Beriberi[1] occurs in the United States principally in hospitals and prisons. It occurs in the Labrador,[2] in Cuba and among the surrounding islands, in the great river regions of South America, the Amazon and Oronoco. In the Orient it is found in China and in parts of other countries in which food is of a limited nature, or has been shipped from long distances. Wherever beriberi appears it is due to the same cause—the monotonous diet of cereal foods in which certain portions of the grains are lacking. In Japan the use of rice which has had the outer layers of the grain removed, leaving practically nothing but starch, is still wide-spread, but the incidence of "kakke," the native name for beriberi, is far less than in former times. In the Labrador the consumption of white bread was formerly high, and when supplemented or wholly replaced by brown bread in the form of whole-wheat or graham bread, the disease largely disappeared. In the United States since 1880, when the milling of white flour began, beriberi has appeared to some extent where the use of white bread and of a diet inadequate in vitamin B is practised. Such cases have been recognized principally in the hospitals for mental defectives, one manifestation of the disease being nervous.

[1] The term signifies "impotence," and while once used to designate what are now known to be different diseases, at present it is confined to the disease described in the above paragraphs.

[2] Through the humanitarian work of Grenfell (English physician) the ravages of this and other diseases have been lessened.

There are *two forms of beriberi*, (1) *wet*, and (2) *dry*. Both involve a Wallerian[1] degeneration. The wet type is so called on account of the edema which occurs owing to the faulty action of the vasomotor nerves controlling the dilatation of the blood-vessels. The dry type is characterized by the paralysis and atrophy of the muscles, due to the lack of nerve supply. This condition is called multiple or polyneuritis.[2] It is for this reason that the vitamin responsible for the disease is called the antineuritic vitamin.

Cause of Beriberi.—The disease has been attributed to various causes, but it is now known that it is due to a deficiency of vitamin B. As an index to the amount of vitamin remaining in rice after polishing, the phosphorus, estimated as P_2O_5, is used. An exclusive diet of

Fig. 196.—Polyneuritis in pigeon fed an exclusive diet of polished rice for three weeks. Compare Fig. 169, showing recovery.

rice having a phosphorus content below 0.4 per cent. is conducive to the production of the disease. The Japanese navy used the nitrogen content instead of the phosphorus as an index, the increase in nitrogen from 16 gs. (which permit beriberi to develop) to 30 gs. per day obviating the development of the disease. The extra nitrogen was added in the form of soy-bean (page 423), which brought in vitamin B.

Beriberi appeared in Japan at the beginning of her period of expansion in the eighteenth century. In order to maintain large supplies of food capable of being kept for extensive periods of time,

[1] Named for the English physician and neurologist, A. V. Waller; when it is desired to follow the course of a nerve tract in an experimental animal, the tract is cut and degeneration of the lipid myelin sheath of the tract takes place, making it possible to stain the preparation differentially and thus to follow the nerves affected to their ganglia. Certain diseases show this phenomenon, beriberi being one.

[2] Greek *polys*, many, and *neuron*, nerve, *itis* standing as the ending of a word agreeing with the word *nosos*, which means illness; see page 268.

rice was polished.[1] During the foreign wars of Japan foods of this nature were necessary. Later, barley was added to the diet of polished rice and the incidence of the disease lessened conspicuously.

Beriberi affected the Roman army during certain campaigns in northern Africa. Later Napoleon, before undertaking similar campaigns, secured the services of the great French chemist, Berthollet, who invented the method of hermetically sealing the containers of foods carried by the army. Thus the disease was obviated.

During the campaign of the British in the Euphrates region (1914–1918) beriberi appeared among the troops, but at present it does not exist in any army.

Beriberi induced experimentally in the lower animals and in man is essentially[2] identical with the spontaneous disease. A method for demonstrating the disease in birds is given in Exercise 31, Chapter XVII. Extensive human experiments were made by Crowell and Strong[3] on criminals in the Bilibid Prison, Manila. Twenty-nine prisoners were selected for volunteers and were divided into groups:

Group I.—Diet: Polished rice plus special diet plus extract of the polishings. Thirty per cent. developed a mild form of beriberi.

Group II.—Diet: As before, but lacking the extract of the polishings. Sixty per cent. developed beriberi.

Group III.—Diet: Red rice[4] and special diet. Thirty per cent. developed beriberi of a modified form.

Group IV.—Diet: Polished rice and special diet. Forty-five per cent. developed beriberi.

These findings are to be contrasted with the experimental production of polyneuritis as described in Exercise 31. The polishings from the rice do not protect against the disease, nor do they serve as efficiently in curing it as other substances, such as beans or yeast, unless care be taken to prepare the polishings properly and to keep them from deteriorating. Experimentation on a large scale on the

[1] A protid which spoils easily is removed from rice by polishing. A similar protid exists in the wheat germ. This is why wheat flour is degerminated.

[2] There are some characteristic differences, as, for instance, those concerned with the nervous system; in experimental beriberi there is more pain and less plantar involvement.

[3] Philippine Jour. Science, Sect. B., vol. 7, Number 4, 1912. Strong, R. P. (Professor of Tropical Medicine, Harvard University). To B. C. Crowell, M.D., Director of the Laboratories Amer. College of Surgeons, Chicago, the author is indebted for advice concerning questions involving pathology.

[4] Red rice is undermilled rice, retaining the protid layer and some of the pericarp, which contains the salts. It is known in the United States as "brown rice."

inhabitants of the Philippines under the direction of Victor G. Heiser[1] showed how unpolished rice protected against beriberi.

That **beriberi is a vitamin deficiency disease** was proved by Eijkman,[2] although Grijns[3] had demonstrated that experimental polyneuritis could be checked by adding extract of native beans to the diet of polished rice. Funk[4] first suggested the name vitamin for a definite substance or group of compounds capable of preventing avitaminoses.

Interrelations of Vitamins and Internal Secretions.—The fundamental work of McCarrison[5] on experimental animals has shown the effect of avitaminoses on endocrine organs. According to this author, diets deficient in vitamins cause hypertrophy of the adrenals, but atrophy of the thyroid, testicles, and ovary. The thymus is affected, but this organ has not been assigned to the endocrines by all biochemists. The pituitary is not affected. The relation between thyroid and intestinal bacterial flora is demonstrated in these studies, for bacterial infection is favored by hypothyroidism, which, in turn, is induced by diets deficient in vitamin. McCarrison explains the edema of beriberi as due to excess secretion of epinephrin, which increases intercapillary pressure and thus mechanically forces the liquid of the blood into the tissues. Excessive glucid diet with low fat content seems to exert some deleterious effect in beriberi which is explained by McCarrison as occurring through the adrenal glands. This is illustrated in epidemics of beriberi in which subjects who eat the most rice (generally men) suffer first and most intensely. Any glucid, however, produces the same result, for the staples like boiled white potatoes, hominy grits, etc., when used under similar conditions cause the disease.

Pellagra.[6]—This disease is found in the mill villages of our Southern States, where it is of a more dangerous type than anywhere else in the world. It was first described in northern Spain where it existed in the Asturias. It is still found along the river Nero, where

[1] Heiser, Victor G., Rockefeller Foundation, New York, N. Y., who was instrumental in developing the great hygienic measures adopted in the Orient, especially in the Philippines, the result of which has been the elimination of many diseases.

[2] Eijkman, C., Dutch investigator, then working in the Dutch East Indies, now Professor of Hygiene, University of Utrecht, The Netherlands.

[3] Grijns, G. (pronounced "hrins"), working also in the Dutch possessions in the East.

[4] Funk, C., Polish investigator, chemical advisor for the Polish Government at Warsaw.

[5] Page 585.

[6] Italian *pella*, skin, and *agra*, rough. It is customary to designate the unknown principle of the nature of vitamin that some suppose to protect against this disease as "PP" (protective principle).

it is known as the "mal de la rosa." Here the inhabitants live largely on corn (maize). In Italy the name "pellagra" was given by Francesco Frapolli of Milan (1771). In the British Isles the disease has been found even as far north as the Shetland Islands. This practically refutes the theory that the disease is due to eating corn, for corn is practically never eaten by the inhabitants of these districts. Moreover, among the North American Indians, who use corn in large quantities ("Indian corn") in their diet, the disease was unknown until the beginning of the nineteenth century. In the last fifty years, pellagra has been found in the Southern United States among all classes of people and the mortality reached 39 per cent. However, the rate began to fall in 1913 and between 1914 and 1916 fell to 10 per cent.

Symptoms.—The disease is manifested by definite symptoms correlated with the season. In winter the nervous system is affected and neurasthenia develops. As spring advances alimentary disturbances arise (stomatitis, eructation, diarrhea). At the same time the skin becomes "sunburned," but, unlike the typical sunburn, the eruptions are quite symmetrical. They occur on the backs of the hands and on the forearms; also a necklace is formed, known as Casal's necklace.[1] The disease progresses and, ultimately, the neurasthenia passes into psychoses and insanity. It is the latter condition which called the attention of medical men to the disease in the Southern United States. Extensive observational and experimental studies of pellagra have been made by the United States Public Health Service, the Rockefeller Foundation, and by various public and private investigators. Goldberger[2] and his associates made experiments in 1915 on prisoners, and concluded that the disease was due to protid deficiency. The Thompson-McFadden Commission[3] likewise studied pellagra and their conclusions are incorporated in the statements given below concerning the cause of the disease.

The *belief that pellagra is a deficiency disease* is favored by the analogy with other diseases of somewhat similar nature, but no definite proof exists that it is an avitaminosis. The prevalent opinion is that vitamin A deficiency weakens the body and favors the develop-

[1] Casal, G., Spanish physician who first described the disease under the name "Rosy Disease," mentioned above as "mal de la rosa."

[2] Goldberger, J., Director of the Field Investigations, U. S. Public Health Service.

[3] From the New York Post-Graduate Medical School. The personnel consisted of Siler, Garrison, McNeal, and others.

ment of tuberculosis, etc. The following summary for believing the cause to be a deficiency in the diet, especially of the vitamin, is presented.

(1) Pellagra is a disease of poverty and largely of women. This indicates that diets poor in fresh materials are the rule, a condition well known to students of the disease. Poverty limits the use of fresh foods and the woman deprives herself of the choicer portions of the food.

(2) Pellagra subjects live largely on monotonous diets. In the mill sections of our South the diet is largely composed of canned goods, which are well-known sources of avitaminoses.

(3) The relation between the use of corn in the diet and pellagra is explained by the spoiling of corn, in moulding, and in bacterial action which destroys the vitamins.

(4) The low content of the pellagra diet in substances containing vitamin A signifies that the deficiency is due to the lack of that fat-soluble vitamin.

(5) Conspicuous differences in closely contiguous districts regarding the food substances show that food is a factor. Thus, in upper Egypt, where millet, which is rich in vitamin A, is largely used as a cereal food, pellagra does not occur; whereas, in neighboring districts in which Turkish corn, deficient in this vitamin, forms a large part of the diet, pellagra is common.

(6) The islands of Sicily and Corfu were free from pellagra while they grew their own corn (prior to the middle of the nineteenth century), but with the advent of foreign shipments of this cereal, with consequent deterioration of the corn, pellagra appeared.

(7) The increase of pellagra in the United States has been explained by Alsberg[1] as due to the newer method of harvesting corn; formerly the corn was husked and dried, but now it is cut at the base and left in the fodder, which, being green and moist, undergoes decomposition. Moreover, much immature corn is thrown upon the market from crops grown in the northwest.

The Theory of Jobling.—Turning now from the hypothesis that pellagra is of deficiency nature, we come to the important theory of Jobling[2] that pellagra is a photodynamic intoxication, due to fluores-

[1] Alsberg, C. L. (Food Research Institute, Palo Alto, California; photograph, page 426).

[2] Jobling, J. W. (Professor of Pathology, Columbia University, formerly of Vanderbilt University, Nashville, Tenn.), in connection with Lloyd, A. D. (Loyola School of Medicine, Chicago, Ill.), Jour. Amer. Med. Assoc., vol. 80, p. 365, 1923.

cent chemical agents produced by certain types of bacteria or fungi in the intestine. The bacteria or moulds particularly responsible for the disease are favored by a high starch diet. This explains why pellagra has developed among people who live largely on a diet of corn. It also explains why the disease is more intense during the period of the year when the actinic rays of the sun[1] are at their maximum; the skin has been sensitized by the fluorescent substances produced in the intestine. Sensitization to light has been accomplished artificially by administering certain substances, like hematoporphyrin,[2] and manifestations which occur in pellagra on exposure to the sun's rays have been obtained, such as erythema on the neck and hands, and edema. Jobling found that the mould Aspergillus glaucus-repens was present in the feces of many pellagra patients, but absent from the excretions of non-pellagrins. The artificial medium in which the mould is cultivated[3] becomes acid, changes color to a light brown, and is strongly fluorescent.

In conclusion, it must be admitted that *the cause of pellagra is unknown.* The fact that a change in diet of peoples affected with pellagra has resulted in an increase or decrease of the disease, not only in our Southern States, but in other countries, indicates a relation between nutrition and pellagra.[4]

Xerophthalmia[5] is a condition definitely attributable to vitamin A deficiency,[6] and during the Great War this cause was practically demonstrated by the conspicuous increase in the disease among the older children of Scandinavian countries deprived of butter and fats. The younger children were not affected because milk was provided for them, but the older ones had their butter and milk rations cut, owing to the economic desirability of shipping such valuable foods to other countries. During 1918, when the German blockade became effective, and exportation of fats from Denmark was discontinued, rationing of the population began. Each person received a quarter of a kilo of butter per week and since other fats, like pork fat and oleomargarin were not available, everyone ate butter with the result that xerophthalmia practically disappeared from Denmark. Each coun-

[1] Page 650.

[2] Page 383.

[3] The medium of Currie, J. N., Jour. Biol. Chem., vol. 31, p. 15, 1917.

[4] See Vedder, E. B., Arch. Int. Med., vol. 18, p. 137, 1916.

[5] Greek *xeros*, dry, and *ophthalmos*, eye.

[6] Other factors than vitamin deficiency have been suggested by Mori (Japanese investigator, Darien, Manchuria), Amer. Jour. Hygiene, vol. 3, p. 99, 1923. Recently xerophthalmia has been shown to be only one of wide-spread forms of Keratosis (page 643).

try is subject to the appearance of the disease whenever, through economic stress, children are made to depend upon foods deficient or lacking in vitamin A.[1] Experimentally, the cause and conditions involved in xerophthalmia have been determined. Mendel in 1913 found that white rats fed a diet deficient in vitamin A developed xerophthalmia. The progressive stages, as described by Yudkin and Lambert,[2] are:

1. Lacrimation.
2. Photophobia.
3. Drowsiness.
4. Edema of the eyelids.
5. Depilation of the eyelids.
6. Swelling of the lids.
7. Receding eyeball into the orbit.
8. Disappearance of the corneal reflex, when the cornea is touched.
9. Incrustation of the cornea and keratitis.
10. Opacity of the cornea.

The cornea is affected only secondarily and, if vitamin is administered early enough, the sight is unimpaired. These workers conclude that the primary seat of the disease is the lacrimal gland, the cells of which show early changes in vitamin A deficiency. The formation of yellow incrustation is due to the invasion of bacteria, which with the normal functioning of the gland is inhibited. When the gland becomes diseased its secretions are no longer capable of preventing bacterial development and consequently leucocytes accumulate (pus formation) to feed upon the bacteria. Such conditions are similar to the "xerosis" and keratomalacia of the ophthalmologist. They are held in check, or altogether eliminated by feeding a diet rich in vitamin A, such as tomatoes, butter, fresh leafy vegetables (spinach or lettuce), and cod-liver oil. Commercial preparations of milk, such as the evaporated, dried, and condensed forms, retain vitamin A, and hence are preventive of xerophthalmia, but skimmed milk and buttermilk are but slightly so.

Rickets (Rachitis).—Man and his domesticated animals suffer from rickets, but such of the tame animals as return periodically to

[1] Bloch, C. E., Blindness and Other Diseases in Children Arising from Deficient Nutrition, Amer. Jour. Dis. Children, vol. 27, p. 139, 1924. See Fig. 167, page 553.

[2] Yudkin, A. M., and Lambert, R. A. (Yale University), Proc. Soc. Exp. Biol. and Med., vol. 19, pp. 375, 376, 1922. Also Jour. Amer. Med. Assoc., vol. 79, p. 2206, 1922.

their wild habits, like the cat, are not known to become rachitic. The white rat, largely used for experimental purposes, can be made rachitic only by closely confining it to certain rachitic diets.[1]

Both children and adults are affected, but adult rickets is much less common. It occurs in women leading a life of confinement, similar to that of the high-caste Mahomedan women who, after marriage, seldom leave their homes. The time of development of the disease

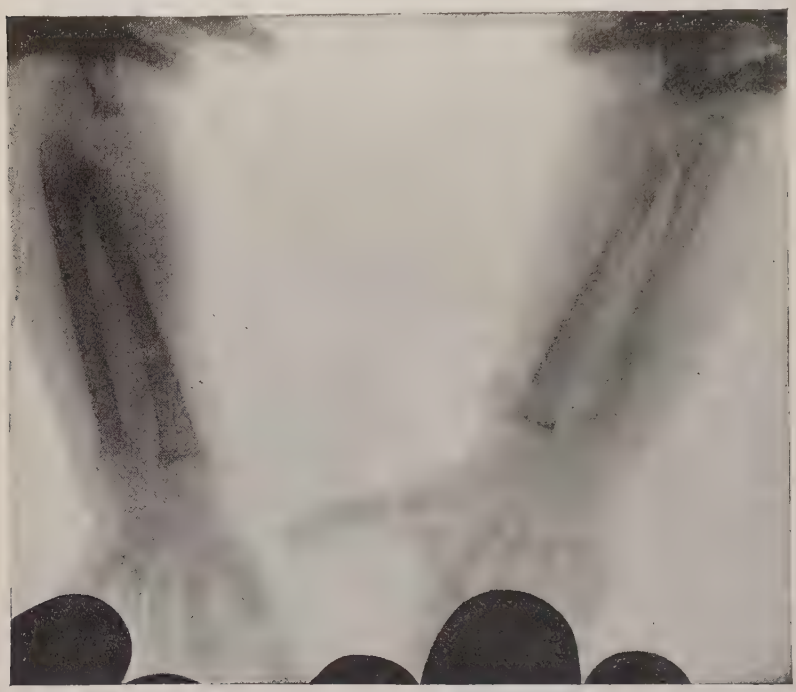

Fig. 197.—Moderate rickets. Compare with Fig. 198. (Alfred F. Hess in Abt's Pediatrics, vol. ii.)

varies. It may be present at birth. Cases have been observed in which the disease developed in spite of the fact that the child was kept in conditions not conducive to rickets. This points to a prenatal factor, perhaps to the condition of the mother. Rickets may be said to be a characteristic condition of prematurely born children. Its incidence is between November and May.

The Disease is Attributed to Avitaminosis.—Formerly vitamin A was assigned as the especial antirachitic vitamin, but recent work has shown the desirability of assigning a specific factor, termed "X"

[1] Page 881.

by McCollum and by Park. Absence of light of a wave-length of about 3000Å[1] is one potent factor for we know that the disease may be treated by subjecting the patient to light of this value, whether derived from natural or from artificial sources. The substance "X" is closely associated with vitamin A and consequently the two have been confused. The nature of this antirachitic substance or its precursor (called by Windaus "provitamin") has apparently been solved by Rosenheim,[2] who finds that not cholesterol but an accompanying sterol, ergosterol, first isolated from ergot, is the active sub-

Fig. 198.—Healed rickets. Daily irradiation for one-half hour with carbon arc lamp. (Alfred F. Hess in Abt's Pediatrics, vol. ii.)

stance. It is destroyed by bromin and by oxidation. It occurs associated with cholesterol and phytosterols. By fractional distillation (Windaus) and by fractional crystillation (Heilbron, et al.) the provitamin has been concentrated to ten times the original amount. It is precipitated in association with digitonin. It is highly unsaturated having 3 bonds. Its discoverer, Tanret, found it to be very sensitive to light and air. Its 3 double bonds make it more sensitive to light than the single one of cholesterol. The characteristic absorp-

[1] Page 133.

[2] Rosenheim, O. and Webster, T. A. Biochem. Journ., vol. 21, page 127, 1927. Also: Lancet, vol. 212, page 307, 1927.

tion is the same as that of cholesterol, namely 2800Å, but the intensity is 2000 times as great. A daily dose of $\frac{1}{250}$ mg. of irradiated ergosterol cures and prevents rickets in experimental rats, while $\frac{1}{1000}$ mg. protects in certain cases. While Tanret obtained ergosterol first from ergot, it is obtainable from many sources (yeast, etc.).

One of the characteristics of rickets is the alkalinity of stools as Zucker has shown. Experimentally, such stools may be obtained by feeding a rachitic diet such as that given on page 881 (Steenbock's). The alkalinity of the stools runs to about *p*H 7.5 and may be reduced to acidity by different means: (1) By the administration of ammonium chlorid (2) by diminishing the Ca/P ratio of the diet, or (3) by irradiation with effective ultra-violet rays ($\mu\mu$ 290), by feeding irradiated sterols or by administering cod-liver oil insured regarding its antirachitic potency. We have referred above (page 559) to the possibility that a primary factor in rickets may be the inability of the intestine to absorb calcium. This may be correlated with the alkaline stools.

The Effects of Light[1] *in Rickets.*—It has been known since 1890 to the medical profession and probably still earlier in a popular way that sunlight had a beneficial effect upon the disease. In 1919 Huldschinsky[2] reported that the ultraviolet ray exerted a curative action. He found that the action concerned the utilization of calcium and that this substance in the form of salts was usually deposited under the action of the light and not especially in rachitic regions, showing that the effect was systemic rather than local. Sunlight, unhindered by ordinary glass is quite as effective as ultraviolet light from artificial sources, but the value of sunlight varies according to the season. The carbon filament, quartz mercury vapor lamp, and cadmium spark act similarly to sunlight, but not the tungsten arc lamp. Hess[3] has shown that the light which is effective in rickets has a wave-length of about 300 $\mu\mu$ (3000Å), or shorter, and that it is possible that two factors, wave-length and intensity, are concerned with the efficiency. Subjection of a patient or experimental animal to unobstructed sunlight, or to light from artificial source having a wave-length and intensity of adequate amounts causes a modification of the concentration of certain blood constituents, especially of inorganic salts.

[1] Park (E. A., Professor of Pediatrics, Johns Hopkins University) prefers to speak of "radiant energy" rather than "light," since the effective ray is invisible to the human eye.

[2] Huldschinsky, K. (Polish physician), Zeitschr. f. orthop. Chir., vol. 89, p. 426, 1920.

[3] Hess, A. F. (Columbia University). For bibliography see Park.

White rats on diet No. 761 (McCollum) gave blood calcium, 5.5 mgs. per 100 mls. blood.

White rats on same diet supplemented with cod-liver oil, fourteen days, gave blood calcium, 8.2 mgs. per 100 mls. blood.

White rats on diet No. 618 gave blood phosphorus 2.4 mgs. per 100 mls. blood.

White rats on same diet supplemented with cod-liver oil, five days, gave blood phosphorus 5.7 mgs. per 100 mls. blood.

Rickets has been produced by:

(1) Lowering the phosphorus intake and increasing the calcium. The disease in this case is characterized by low blood phosphorus, lack of ossification, and irregular distribution of osteoblasts.

(2) Lowering the calcium and increasing the phosphorus. The disease is characterized by low blood calcium, but ossification centers are more regularly arranged.

(3) Administration of magnesium carbonate. The disease is characterized by its severity, low blood phosphorus, and general appearance of (1).

(4) Addition of strontium carbonate to the diet. The general effects are similar to (1) and to (3).

The feeding of pasteurized milk produces rickets, apparently through the lowering of both Ca and P in the blood of the animal to which milk is given. The quantitative relations between phosphorus and calcium are reported by Howland and Kramer[1] for blood concentrations as follows: If the quotient,

$$\frac{\text{Mgs. inorganic blood phosphate} \times \text{mgs. blood calcium}}{\text{100 mls. whole blood}},$$

is equal to or above 40, rickets will not develop; if less than 40, rickets will develop.

There is a relation between the development of rickets by a given diet and the acid-base value of the diet. When the rickets-producing diet of Sherman,[2] which contains flour, cheese, calcium lactate, sodium chlorid, and iron as ferric citrate, was made more acid by replacing the lactate with $CaCl_2$, rickets did not develop. Neither light nor vitamin is a factor.

Prevention of Rickets.—It is evident from the foregoing discussion that rickets may be prevented by:

(1) Furnishing foods which contain the proper "vitamin."

(2) Furnishing light of proper wave-length, namely, 300 $\mu\mu$ (3000 Å). Recent work seems to show that the "vitamin" is a chemical

[1] Howland, J., (Johns Hopkins University, deceased) and Kramer, B., (Jewish Hospital, Brooklyn, N. Y.) Monatschr. f. Kinderheilkunde, vol. 24, p. 279, 1923.

[2] See Zucker, T. F., Proc. Soc. Exp. Biol. and Med., vol. 20, p. 20, 1922.

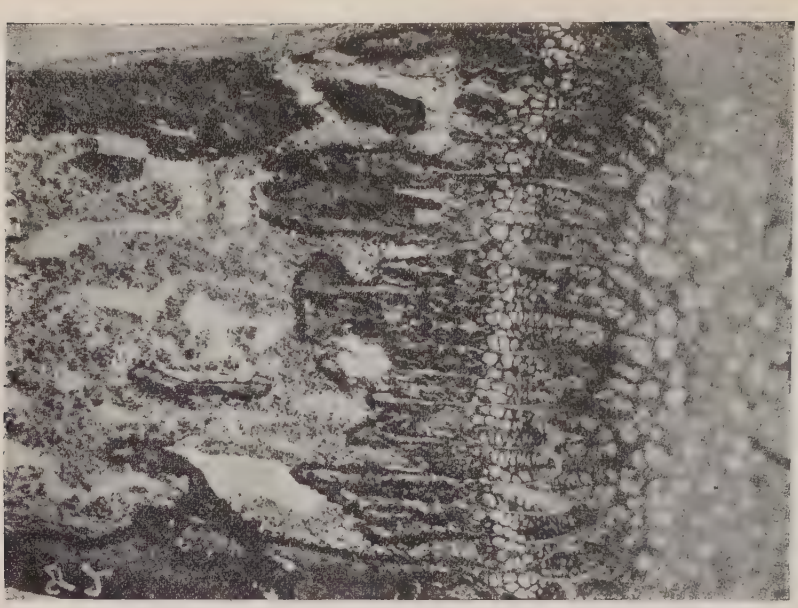

Fig. 199.—Normal rib of rat. Decalcified. Compare with Fig. 200. (Alfred F. Hess in Abt's Pediatrics, vol. ii.)

Fig. 200.—Rib of rat. Thirty-four days on rickets-producing diet. Marked rachitic lesions. Absence of "white line." Note increased width and irregularity of proliferating cartilage, absence of calcium deposition, great excess of osteoid in region of metaphysis and about cortex. Compare with Fig. 199. Decalcified. (Alfred F. Hess in Abt's Pediatrics, vol. ii.)

configuration due to the action of this light. The succesful manufacture of a quartz "glass," known by the trade-name "Vitaglass" and also of a fabric, both permeable to rays of about 2800Å make it possible to build accommodations for children being treated for rickets.

The *effect of irradiating cholesterol*,[1] a constituent of the substances that may be irradiated and rendered antirachitic, can be seen from the following experiment of DeFrates:[2]

ALBINO RATS, WISTAR INSTITUTE STOCK[1]

No.	Sex.	Born.	Diet.	Weight				
				Mar. 28.	Mar. 30.	Apr. 1.[2]	Apr. 22.	Apr. 29.
1.....	♀	Jan. 29th	No. 1	68 gs.	70 gs.	67	76	84
2.....	♂	Jan. 29th	No. 1	48	50	50	60	68
3.....	♂	Jan. 29th	No. 1	67	68	66	79	90
4.....	♂	Jan. 29th	No. 1	78	78	79	80	79.5
5.....	♀	Jan. 29th	No. 1	61	62	61	54 (died)	

[1] Wistar Institute, Philadelphia. The Institute furnishes pedigreed stock for experimental purposes.

[2] Began feeding cholesterol; Nos. 1, 2, and 3 fed cholesterol, irradiated fifteen mins. Distance 2.5 dms. Nos. 4 and 5 not irradiated.

The diet used in these experiments (Diet No. 1) consisted of the following substances (this is practically the diet of Steenbock, for which the author is indebted to Dr. Steenbock):

	Portions.
Yellow corn	76
Wheat gluten	20
$CaCO_3$	3
NaCl	1

It is evident that rats fed irradiated cholesterol (Nos. 1, 2, and 3) grew at normal rates, while rats fed cholesterol which had not been irradiated, failed to grow, or died (Nos. 4 and 5). The diet in all cases was such as to develop rickets, unless a second factor were furnished; this accessory factor was irradiated cholesterol. It is interesting that DeFrates was unable to obtain curative effects from phytin (page 374), which has a chemical configuration somewhat similar to cholesterol, but lacking a terpene radicle (page 217).

In another set of experiments DeFrates showed that irradiation does not produce any recognizable chemical change in irradiated foods, such as olive-oil, and while others, like Hess, have reached a similar conclusion, it does not follow that the light does not alter the constitution of these food substances in some way as yet not discovered.

[1] See page 645 regarding ergosterol.

[2] DeFrates, J. S., data unpublished. See Preface to First Edition.

While excellent results in the control of rickets have been obtained by the use of artificial light, sunlight, when used through unobstructing transparent quartz or from the sun in the open air, have yielded

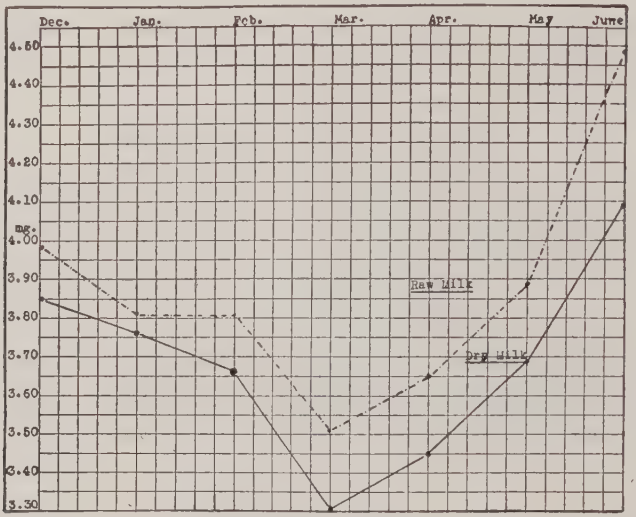

Fig. 201.—Seasonal variations in inorganic phosphate of the blood; diet, milk. Compare with Fig. 202. (Alfred F. Hess in Abt's Pediatrics, vol. ii.)

similar favorable consequences. Such transparent quartz window-panes are being installed in hospital solaria throughout the country.

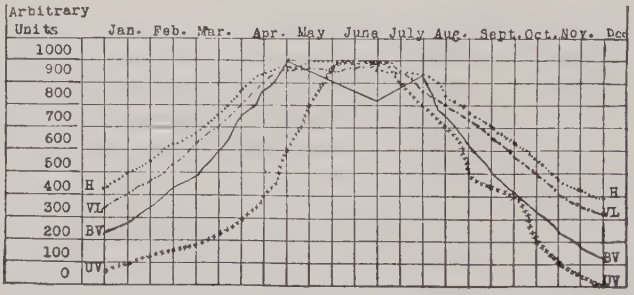

Fig. 202.—Seasonal variations of the sun's spectrum: *H*, Solar heat; *VL*, visible light; *BV*, blue violet; *UV*, ultraviolet (Alfred F. Hess in Abt's Pediatrics, vol. ii.)

Over-illumination has been found to be deleterious, but Leigh-Clare (Biochem. Journ., vol. 21, page 208, 1927) attributes the difficulty to conjunctivitis.

Tetany, which will be discussed in detail later, in the present Chapter bears a certain relation to rickets and frequently accompanies this condition. Calcium metabolism is concerned in both instances; both are diseases encountered in prematurely born children; both show seasonal variations. Tetany, as we shall see, probably has its cause in the parathyroid gland, in the absence or insufficiency of which calcium metabolism is affected. Tetany is induced by parathyroidectomy,[1] by sodium poisoning on feeding Na_2CO_3, or $NaHCO_3$, and by gastritis. The blood calcium is reduced, whereas the blood phosphorus remains normal. Calcium administered as $CaCl_2$ improves tetany in children and the blood calcium rises, with a concomitant fall in phosphorus. Infantile tetany treated by means of the mercury vapor lamp improves clinically and there is a return to normal of blood calcium; the blood phosphorus also remains normal. In general, infantile tetany responds to the same curative measures that are used in the treatment of rickets. By using a method similar to that which he used in purifying insulin, Collip[2] succeeded in isolating a fraction of parathyroid material which, when administered, relieves all symptoms of the disease in parathyroidectomized, experimental animals (Figs. 205 and 206).

Sprue[3] is a disease typically of the tropics, characterized by a catarrhal inflammation of the mouth (stomatitis) and alimentary tract. It seems to be aggravated by a low protid diet and one excessive in fat.[4] There is constantly associated with the disease an intestinal parasite belonging to the fungi, known as Monilia. Authorities disagree concerning the relation of this plant to sprue. Fresh milk[5] is used as treatment, but it is not known whether a vitamin is concerned or whether the increased amount of calcium administered by way of the milk is responsible. Administration of calcium in the form of calcium lactate and parathyroid tissue is also beneficial

[1] Sometimes in removing the thyroid in goiter cases the parathyroid is disturbed, or is accidentally removed, and tetany results; the condition is called tetany strumipriva, from the Latin *struma*, goiter, and *priva*, to deprive of.

[2] Collip, J. B. (page 660), Jour. Biol. Chem., vol. 63, pp. 395, 439, 1925.

[3] Sometimes called "sprew," "thrush." The term "sprew" is a Dutch word, modified from the native word "spruw" of the Dutch East Indies. It occurs in the United States (Wood).

[4] Although high fat diets act unfavorably, there is a fat deficit in an untreated case of sprue. Milk is almost a specific for the improvement of sprue.

[5] For an account of the diet of sprue patients in Porto Rico, see Ashford, B. K., Amer. Jour. Trop. Med., vol. 2, p. 139, 1922. The diet is increasingly "rice, polished and imported."

in this disease. Current opinion tends to favor a dual origin for the disease: (1) Food deficiency, and (2) infection by the fungus, Monilia.

Scurvy was first scientifically studied in Norway. The sailors of that country experienced the ravages of the disease on their fishing voyages. The early experimental studies of the two Oslo[1] investigators, Holst and Fröhlich[2] were stimulated by the reports of the investigations of Eijkman on beriberi and experimental polyneuritis, described on page 639. Using guinea-pigs, they repeated Eijkman's experiments with polished rice and found that, unlike pigeons which showed symptoms of polyneuritis on such a diet, guinea-pigs developed scurvy, identical, as far as symptoms were concerned, with the human disease.[3] Before this systematic work of the Scandinavian investigators, it had been noted in the United States by Theobald Smith[4] that an exclusive diet of oats produced scorbutic lesions in guinea-pigs. The Oslo workers found, later, that bread and other cereal preparations gave similar results. As antiscorbutic substances, they used fresh, uncooked cabbage, carrots, dandelion "greens," lettuce, endive, potatoes, and fruits like bananas and apples.

Stefánsson[5] found that he could live for years in the north countries on meat diets without contracting scurvy, if blood, raw meat, and glandular tissues like liver and kidney were used. One of his men, who became impatient with the progress of the expedition, sulked, and refused to eat raw meat, developed scurvy. This, however, disappeared on resumption of a fresh meat diet, especially one including blood. The success of James Cook who circumnavigated the world in 1768 was largely due to his insistence that the crew eat 20 lbs. of onions per man per week, which were cooked with grasses gathered at ports of call.[6]

During the Great War scurvy affected the troops in spite of our knowledge of its etiology and curative measures. In Mesopotamia in 1917–20[7] scurvy affected the troops from India, and beriberi the

[1] The current name for Christiania.

[2] Holst, A., and Fröhlich, T. (University of Oslo, Norway), Jour. of Hygiene (English), vol. 7, p. 619, 1907.

[3] The chief symptoms are given in the legend beneath the figures showing the manifestations of the disease.

[4] Smith, T., Rockefeller Institute for Medical Research, Department of Animal Pathology, Princeton, N. J.

[5] Stefánsson, V., Anthropologist and Arctic traveler. See My Life with the Eskimos. Also The Friendly Arctic, both published in New York, The Macmillan Co., 1913 and 1921.

[6] See Nat. Geogr. Mag., vol. 51, page 85, 1927.

[7] Page 638.

British. The British soldier is frequently called the "lime-juicer" on account of his faithful attention on expeditions to a supply of the juice of the fruit of the lime, but the campaign was of such a nature that this supply failed.[1] The expedition of Shakelton[2] into the Antarctic was kept free from scurvy by the use of concentrated lemon-juice and tablets from the same source.

Tomatoes protect against scurvy in guinea-pigs in amounts of from 1.5 to 2.5 mls. fresh juice. Canning reduces potency 75 per cent.; bottling reduces the potency 60 per cent. Once prepared, however, the canned or bottled preparation remains unchanged for years. Freezing reduces the potency not only of tomato-juice, but of all antiscorbutic substances.

The use of the white potato[3] by the Germans and by the Irish saved these countries during times of stress from outbreaks of scurvy, and the same may be said of parts of the United States. The most dependable antiscorbutic agent we have at the present time is the tomato, which, for years, was not considered as food, being known as "love-apple" and fit only for display purposes. With the increase of hermetically sealed and sterilized foods, the tomato stands out clearly as an antiscorbutic substance, retaining its protective properties under severe conditions.

Infantile scurvy[4] is produced by the use of milk preparations such as evaporated, condensed, etc.[5] Wherever high temperatures are used, as in evaporating milk for the market, the antiscorbutic value is reduced or altogether lost.

At the present time foods deficient in vitamin C in use in Europe and in Asia are conducive to the development of scurvy. In 1923 Prussia had 386 unmistakable cases of scurvy, although the disease had not been observed there previous to 1915. Undernutrition and

[1] Lime-juice loses its potency as an antiscorbutic agent after a time and this has been the cause of many serious outbreaks of scurvy in various expeditions. The so-called "lime-juice" of the British Army of earlier times was actually lemon-juice. The potency of lime-juice is remarkable when fresh and when concentrated with only 0.07 per cent. solids still shows its original activity, but its power is lost on standing.

[2] See the report of the surgeon of the expedition: Macklin, A. H., and Hussey, L. D. A., The Lancet (English Journal), vol. for 1922, second part, p. 322. Scurvy appeared with fatal results in the expedition of Scott to the South Pole, due, apparently, to the failure of the antiscorbutic preparations provided for the usage of explorers.

[3] See Appendix for the contrast in antiscorbutic value of the white and sweet potato.

[4] Barlow's disease (Sir Thomas Barlow, British physician of the nineteenth century).

[5] Attempts have been made to show that milk preparations contain adequate antiscorbutic properties. Undoubtedly, a small amount of such substance is present, but that, as the milk preparations are used they are protective, is not borne out by facts. This statement does not apply to all forms of milk preparations.

avitaminosis are reported to involve increased basal metabolism, and the feeding of vitamin C diets results in the decrease in oxygen consumption to normal. Much scurvy goes undetected in its incipient form, being mistaken, owing to the edema for cardiorenal disease. The error is discovered only after careful clinical study.

The *relation between vitamin C deficiency and decayed teeth* is of especial importance. Although advertisements would lead one to believe that the modern mouth is diseased, while that of our ancestors was perfect, the actual facts show this to be untrue. The teeth of mummies and of other human remains are frequently in a condition similar to that of the worst mouth encountered by the Twentieth Century oral surgeon. In Egypt diseased teeth were probably the result of the diet restricted to cereals, like maize (corn), which is lacking in vitamin C. There is no question that teeth suffer in cases in which the diet is low in the antiscorbutic vitamin. For the legend "A clean tooth never decays" we may well exchange "A vitamin-fed tooth never aches."

ENDOCRINOSES

The endocrinoses include such pathological states as are attributable to endocrine disturbances. We have seen, in the foregoing section, that the vitamins exert a profound influence upon the organism; even when present in small amounts, they change the course of metabolism. The endocrines likewise exert a great influence on the body and, as we shall see, minute amounts determine whether a certain metabolic condition shall exist. On the other hand, diseases of the endocrine organs occur which are not due to the deficiency of the secretion, but to other causes, few of which are known. Endocrine substances are produced within the human body; the chemical basis, however, must be derived from plants. The influence of internal secretions is manifested principally through the sympathetic nervous system. Here, as in the case of the vitamins, our knowledge of the biochemistry involved has been derived largely through the study of pathological states affecting the endocrine glands. The reader is warned, however, that the correlation between endocrines and disease is not definitely known in many cases and that there are many differences of opinion.

Goiter.—This is a more or less popular term, which is rather loosely applied to various diseases of the thyroid[1] gland. Associated with goiter are states: Due to a deficiency in the secretion of the thy-

[1] Greek *thyreos*, a shield; so called from the shape of the gland, Greek *eidos*, like.

roid gland, known clinically as hypothyroid conditions; but those due to an excess of the secretion of the gland, known clinically as hyperthyroid diseases are strictly "goiter." We have discussed the chemical substance responsible for the particular biochemical characteristics of this gland,[1] thyroxin, discovered by Kendall. The normal requisite of thyroxin is about 0.6 mg. per day. Deficiency in this substance causes the following diseases:

Hypothyroid Diseases.—*Cretinism* (so named because of its prevalence in the Island of Crete off the southern coast of Greece, a mountainous island, with valleys supplied with water from mountain streams) develops before puberty. Dwarf and deformed children are the results of cretinism. If taken sufficiently early, cretinism may be cured by the administration of iodin in some form. Cretinism may be called "infantile myxedema." Two phases of the disease are recognizable:

Fig. 203.—E. C. Kendall, Mayo Foundation, Rochester, Minnesota. Discoverer of thyroxin.

(1) Myxedematous type: Persistent lack of development of the sex-organs, dwarfism, mutism, muscle inco-ordination, and low mental development are characteristic.

(2) Nervous type: Tetany, idiocy, bilateral paralysis (diplegia), and hyperexcitability of the nervous system. In the Himalayan regions, in the valleys of the Swiss, French, Italian and Austrian mountains, and throughout the globe, where similar topographical conditions prevail, cretinism is endemic. At puberty: This form called, variously, endemic, cystic, simple, adolescent, etc., is seen with the greatest frequency in our Middle West, especially in a belt extending from Old Smoky in the Southern Appalachian Mountains to Winnipeg, Manitoba, Canada. It is largely confined to the female subject. During menstruation, and other sexual periods, the size of the thyroid gland becomes greater. The basal metabolic rate is normal. The disease rarely involves other organs, but the goiter later becomes adenomatous and degenerative, fibrous or calcareous changes

[1] Page 271.

occur. Actual exophthalmic goiter may develop later in life. Iodin is now administered to the inhabitants of affected regions, sometimes in the form of phials of iodid, given to school children, or sometimes the iodid is distributed in the form of table salt, or through drinking-water, in the municipal supply. Table salt containing iodin is now available on the market.[1] The response to such treatment is rapid. A well-developed goiter, however, never completely retrogresses.

Myxedema[2] is observed in adolescents and adults. It is characterized by edema of the face and hands, with accompanying dryness and wrinkling of the skin. Low basal metabolic rate, slow pulse, loss of mental alertness, and loss of hair from the body are the usual symptoms. Administration of thyroxin produces an increase in B. M. R. and a return of the skin condition to normal. Administration of thyroxin or of iodin must be made with care,[3] for overdoses lead to conditions resembling the following group of hyperthyroid diseases, especially in the adenomata of later life, which may follow simple goiter or myxedema.

Hyperthyroid Disease.—*Basedow's*[4] *disease;* exophthalmic goiter; toxic goiter; Graves'[5] disease. This disease is characterized by the increase in B. M. R., increased pulse-rate, exophthalmos,[6] nervousness, etc. Conditions similar to exophthalmic goiter appear following overdoses of thyroid preparations used to reduce obesity. The sympathetic nervous system is affected. The treatment is "skilful neglect" by permitting the patient to rest under controlled conditions; surgical excision of the thyroid gland, or ligation of its blood-supply; and regulation of the diet. Administration of definitely known quantities of thyroxin, or of iodin preparation, to exophthalmic goiter patients improves the condition, even within short intervals of time,[7] but must not be carried too far. The toxicity involved is similar to that induced by overfeeding of thyroid or of iodin; these effects are not due to the direct effect of thyroxin. This agent accelerates metabolism throughout the body and it is this extra metabolism whch produces the toxic manifestations.

[1] Preparation distributed by the Morton Salt Co., Chicago, contains 0.00002 g. iodin per 100 gs. of the salt.

[2] Greek *myxa*, slime, and *oidema*, a swelling.

[3] See editorial, Therapeutic Gazette, vol. 49 (W. S.), p. 265, 1925.

[4] Basedow, K. A. von, German physician of the eighteenth century.

[5] Graves, R. J., Irish physician of the eighteenth century.

[6] Greek *ex*, out, and *ophthalmos*, eye; protruding eye. The cause of the protrusion is unknown.

[7] Boothby, W. M. (Mayo Foundation), Endocrinology, vol. 8, p. 725, 1924.

Adenomatous goiter[1] is distinguished from true exophthalmic goiter by the characteristic manner of responding to thyroxin. Favorable effects are obtained by administering thyroxin to hyperthyroid cases, but unfavorable when given to patients suffering from adenomas with hyperthyroidism. The latter is characteristic of persons up to about thirty-five years, while adenomatous hyperthyroidism is generally of later occurrence.

Iodin and Goiter.—Disturbances of the thyroid gland are general, but the incidence of goiter is greater in certain regions than in others in which there is a lower content of iodin in water and foods.[2] In the latter districts the iodin content of drinking-water varies from 1 to 22 parts of iodin per 100,000,000,000 parts of water, while in the non-goitrous regions the iodin content of the water supplying the districts is more than 23 parts per $(10)^{11}$ parts H_2O. The two maps represented on page 584 illustrate the coincidence of low iodin content and high goiter. The difference in iodin content of food materials grown in non-goitrous and in goitrous districts is, as a rule, remarkably correlated with the iodin content of water in these districts. Thus, from non-goitrous regions, like certain parts of New England, the iodin content of wheat estimated as milligrams per metric ton[3] of wheat varies from 4 to 10, while wheat from goitrous districts (Minnesota) contains from 1 to 7. Carrots, from non-goitrous regions in California, have an iodin content of 170 contrasted with 2.3 in carrots from the goitrous districts of Oregon.

Addison's Disease.[4]—We have discussed the chemistry of epinephrin,[5] which is produced in the adrenal glands. Of the two histological portions, (1) the outer cortical and (2) the inner or medullary zone, the second is of especial interest in Addison's disease, although both portions are affected. This medullary zone consists of elements derived from the nervous tissue belonging to the sympathetic system and, primitively, the cells are differentiated into (*a*) sympathetic, multipolar nerve cells, and (*b*) cells which later

[1] For a discussion of adenomatous goiter and hyperthyroidism, as well as the use of thyroxin in goiter, see Boothby, W. M., Endocrinology, vol. 8, p. 727, 1924.

[2] For a discussion of the relation between goiter and iodin in food and water see McClendon, J. F. (Minnesota), Inverse Relation Between Iodin in Food and Drink and Goiter, Simple and Exophthalmic, Jour. Amer. Med. Assoc., vol. 82, p. 1669, 1924.

[3] Metric ton = 1000 kgs.

[4] Addison, T., English physician of the early nineteenth century.

[5] Page 267.

become the "chromaffin cells,"[1] with which the internal secretion of the adrenals is concerned. It is this portion of the gland which becomes defective in Addison's disease.

The *characteristics of the disease* are hypoglycemia, pigmentation of skin, hair, eye-lashes, etc., giving a bronze color; nervous symptoms, such as insomnia, headaches, later delirium and convulsions, ready fatigue, apathy, rheumatoid pains in the back, and elsewhere; low blood-pressure, adrenal hypoplasia in many cases;[2] cardiac atrophy, atrophy of the sex organs. The disease is frequently associated with tuberculosis, but is a condition of middle age, being rare in children and in the aged.

The *cause of the disease* is attributed to the loss of function of the adrenals. Experimentally, many of the symptoms of the disease may be induced when the adrenals are removed. Stewart and Rogoff[3] find that when the adrenals are removed from experimental animals (dogs), the most pronounced symptoms are the profound disorganization of the digestive system, weakness only toward the latter stages of the survival before death, hyperexcitability of the nervous system, circulatory disturbances and, at autopsy, hemorrhage of the gastrointestinal tract. The duration of life after adrenalectomy extended from 3.5 to thirty-four days, the longer periods being in dogs that were injected with Ringer's Solution,[4] to which some glucose had been added. Since these beneficial effects were obtained without the administration of any endocrine material, it is scarcely to be expected that the gastrointestinal and other symptoms mentioned above are due to any disturbance of the endocrine system.

Experimentally, it is nearly impossible to remove either the cortex or the medulla alone. Consequently, the knowledge that we desire as

[1] Literally, the "colored cells," because chromic acid turns them brownish, and ferric chlorid, green. These cells occur in nests and are found not alone in the adrenals but also in the carotid artery, ganglia of the sympathetic trunks, solar plexus, left stellate ganglion, junction of the left coronary artery and the superior mesenteric, hilus of the kidney, and along the course of the sympathetic nerves. There may be compensation on the part of the other portions of the chromaffin system for loss of function of the chromaffin cells in the adrenals and, therefore, there may be no Addison's disease following destruction of the medulla.

[2] This condition has been observed where there is no evidence of Addison's disease. This may be explained by considering that chromaffin tissue outside the adrenal glands may take over the function of the chromaffin tissue of the adrenal, but few studies have been made to determine this point.

[3] Stewart, G. N., and Rogoff, J. M. (Western Reserve Medical School, Cleveland, Ohio), Proc. Soc. Exp. Biol. and Med., vol. 22, p. 394, 1925.

[4] Page 401.

to the results of the loss of function of either of these portions of the glands has never been obtained.

Treatment by the administration of epinephrin has led to no proof that Addison's disease is due to the lack of this substance.[1] Whole-gland tablets, when administered, have produced a reduction of pigmentation and it is doubtless the cortex which is the principal factor. The cortex is derived from the same general portion of the embryo that the sex cells are, and injury to the cortex, as, for instance, by a tumor, produces marked effects upon the secondary sexual characters (lack of hirsuteness, defective maturity of the external genitals, etc.). Administration of epinephrin by mouth is generally ineffective.[2] Subcutaneous injections cannot be maintained for long periods.

We cannot say definitely that Addison's disease is caused by the lack of any internal secretion, especially epinephrin, but, on the other hand, this cause has not been shown to be ineffectual.

Next will be considered certain conditions which are believed to be due to a lack of some specific endocrine substance, although the correlation is difficult.

Tetany Parathyroprivia.[3]—Tetany is an abnormal hyperexcitability of the nervous system attended by systemic conditions, such as alteration of the inorganic constituents of the blood. In all animals damage to the parathyroids, especially in the young subject, induces tetany, but the effect varies with the species. The outstanding feature seems to be the development of a toxic condition, and this has led to the theory that the function of the parathyroid is that of a detoxicating gland.[4] The most efficient treatment hitherto has been the administration of calcium, which is low in the affected organism.

We do not know the nature of the toxic agent, but the administration of methyl-guanidin, which is creatin, deprived of the acetic acid radicle,[5] has been found to exert an influence similar to that of the removal of the parathyroids. Tetany is controlled by lowering the

[1] See Wells, H. G., cited on page 346. Since Kendall (personal communication 23 Dec., 1926) has found that a catalyzer of epinephrin is necessary for its action, it might be possible that in Addison's disease, such a catalyzer is not present.

[2] Patients with Addison's disease are benefited by epinephrin, but the reason lies in its vascular action. Epinephrin is favorable to other diseases which are not endocrinoses, such as asthma.

[3] Page 259.

[4] This function has been assigned to many other glands, thyroid, adrenal, and others. See page 143.

[5] Page 364.

absorption of toxin from the alimentary canal and by inducing its elimination. There is some connection between tetany and a diet of meat,[1] which probably is due to the creatin of this food. Treatment by the use of parathyroid extracts has previously led to no improvement, but recently Collip[2] has secured an extract from parathyroids which acts favorably in the treatment of tetany.[3] This work is the first positive demonstration of the dependence of tetany upon para-

Fig. 204.—J. B. Collip, Professor of Biochemistry, University of Alberta, co-receptor of the Nobel Prize; initiated the preparation of insulin in practical amounts and isolated the hormone of the parathyroid gland. Earlier work on the origin of gastric hydrogen chlorid.

thyroid inefficiency. The relation of calcium to tetany is known only in an empirical way, but Collip finds that his extract causes a rise in blood calcium. He defines the potency of the parathyroid extract in units, each unit being one one-hundredth of the amount of extract which causes an increase within twenty-four hours of 5 milligrams of serum-calcium in a 20-kilogram parathyroidectomized dog. The

[1] Collip (see above) is able to feed experimentally parathyroidectomized animals into which an extract of parathyroid tissue has been injected, with high meat diets.

[2] Page 24. See Collip, J. B., and Clark, E. P. (Alberta), Jour. Biol. Chem., vol. 4, p. 485, 1925.

[3] Excessive doses of the extract cause overabundance of calcium in the blood, with consequences of pathological nature, such as vomiting, cardiac involvements, etc.

Fig. 205.—Dog fifty-nine days after removal of thyroid and parathyroid. Tetany parathyroprivia. (After Collip, Jour. Biol. Chem., vol. 63, p. 400, 1925.)

Fig. 206.—Same dog as shown in Fig. 205, three hours after subcutaneous injection of Collip's parathyroid extract. (From Jour. Biol. Chem., vol. 63, p. 400, 1925.)

greatest increase of calcium thus far obtained is up to 20 mgs. per 100 mls. blood-serum. Alter the calcium has risen to a certain level phosphorus begins to rise in the blood likewise.

Tetany does not necessarily occur in simple parathyroid lesions. Rarely, it complicates goiter when the latter involves the dysfunction of the parathyroids. Tetany also accompanies certain infectious diseases, especially typhoid fever. In tuberculosis involvement of the parathyroid may lead to tetany. Poisons, like morphine, may induce it. It occurs among persons following certain trades, such as shoemaking, repairing, and tailoring. It is typically a disease of the city. McCarrison has reported the coincidence of tetany and goiter of myxedemic type, but tetany does not seem to be associated with ordinary colloid goiter. In fact, the disease is quite rare in the Alps, Tyrol, and in other goiter districts. Tetany in children seems to be related to lesions in the parathyroids, for hemorrhages occur in these glands during the course of the disease. Various gastro-intestinal diseases may cause tetany. Hyperparathyroidism, involving adenomata, has been observed. As a rule, no correlation is evident between excessive growth of the parathyroid and any bodily dysfunction.

Gonadal Diseases.—Under this heading are grouped diseases which are connected with lesions of certain internal organs and which affect the genital system, either the primary male and female sex cells, or the secondary sexual characters, such as hirsuteness, shape of the body, pitch of the voice, etc. We have already mentioned the relation existing between the genital organs and the adrenal cortex.[1]

Previously, it was believed that there is a relation between the thymus gland and other glands of internal secretion which control reproduction and growth. Feeding thymus substance to larval frogs previous to metamorphosis delays the process and the tadpoles become enlarged. Certain pluriglandular disorders (Timme's syndrome[2]) have been referred to the thymus reacting with other glands, but in no case has it been proved that an internal secretion is formed by the thymus. Certain work indicates that an association exists between thymus and the hypophysis, about to be discussed. Whatever the nature of the thymus may be, it is evident from the extensive investigations of Park and McClure[3] that the thymus is not

[1] Page 659.

[2] Timme, W. (New York neurologist), Med. Clin. of N. A., vol. 2, p. 959, 1919. Pluriglandular diseases will be discussed later (pages 669 and 671).

[3] Park, E. A., and McClure, R. D. (Chief Surgeon, Ford Hospital, Detroit, Mich.), Amer. Jour. Dis. Child., vol. 18, p. 317, 1919.

indispensable to the dog, or probably to any mammal, including man. Incidentally, the administration of mixtures of hormones ("pluriglandular therapy") has not been found to have any distinctively favorable effects.

Premature sexual development has been correlated with lesions of the epiphysis.[1] Both structural and functional disturbances involving the higher nervous centers occur in the sexual system. The gland enlarges and pressure is exerted on the surrounding brain tissue. In older persons, adiposity seems to be produced. In the young, there is an accelerated sexual development, especially in the male; premature deepening of the voice, enlarged intromittent organ, precocious estruation, and other manifestations are noted. Both the administration of pineal substance and the destruction of pineal tissue leads to precocious development. However, here, as before, much more information is necessary before a definite rôle may be assigned to the pineal apparatus or its relation to the development of the genital system proved. No internal secretion has been detected, all evidence being physiological.

Dystrophy adiposogenitalis, or *eunuchoidism*, occurs when the hypophysis[2] is injured or removed. There is an accumulation of fat over the body, but true obesity does not result; subjects affected with this disease do not respond to regulations of diet and other treatments designed to reduce obesity. There are three factors recognized in this condition: (1) Hypophysis; (2) interstitial cells of Leydig in the testis, and (3) thyroid. Since subjects with known lesions of the hypophysis do not always develop such form of dystrophy, it cannot be ascribed to this organ alone in all cases. The same may be said respecting the

[1] Greek *epi*, upon, and *physis*, nature, or substance. The organ lies over the third ventricle of the brain and is derived from that region, the primitive end of the central nervous system. It becomes infiltrated with connective tissue from the seventeenth year of life. It reaches its greatest development about the seventh year. The structure is sometimes called the pineal apparatus.

[2] Greek *hypo*, under, and *physis* as in epiphysis. The organ is dual in origin: (1) Neural, an evagination from the floor of the brain comes into contact and slips by (2) an upward evagination from the alimentary canal, in the region of the pharynx. The pharyngeal lobe lies forward of the neural; this anterior lobe is epithelial in nature, the posterior lobe being filled with neuroglia. The stalk connecting the anterior lobe with the brain is known as the infundibulum. Pituitrin sold on the market as "pituitary substance" or "extract" comes from the posterior lobe, but Biedl (famous Czech pathologist) believes that the substance is made from the pars intermedia, lying immediately posteriorly to the anterior or pharyngeal lobe. The substance is then transferred to the posterior lobe, where it is stored, or tarries on its way to the third ventricle of the brain, where it mingles with the cerebrospinal fluid. Recently Abel at Johns Hopkins University has partly purified this substance by means of picrate and tartrate. See page 667.

gonads; eunuchs do not all become overfat. Again, the thyroid may show no derangement, judged chiefly by the normal basal metabolic rate. It is certain in many cases that the hypophysis has something to do with the condition, for feeding by mouth of hypophyseal material produces favorable results. Reports have been made that such treatment plus administration of sex gland substance acts favorably.

Adipositas dolorosa was first described by Dercum[1] and is known as Dercum's Syndrome.[2] It is characterized by a deposit of fat on the shoulders, deltoid region, chest, pelvic region, and abdomen. The forearms, arms, forelegs, and legs remain the natural shape and size. Dercum ascribes the disease to a dysfunction of the thyroid gland. The hypophysis, adrenals, and sex glands are normal. Administration of thyroid preparations has a favorable effect. The condition is described here in order that it may be differentiated from the foregoing states which involves fat deposition.

Late eunuchoidism[3] is the technical designation of a condition which is characterized by fat deposition, especially on the mons veneris, breast, and hips with accompanying atrophy of the genital apparatus, both primary and secondary. In the male the penis, scrotum, prostate and secondary sexual characters, such as the beard; in the female, the labia majora and the uterus, retrogress after having functioned for several years. Tuberculosis of the genital apparatus, trauma, as in war, or any cause inducing the atrophy of the sexual apparatus is responsible for the disease. The interpretation of reports of gland administration in the treatment of this condition has to be made with great care, because psychic effects enter into the matter; a depressed mental state due to hypofunction of almost any portion of the body is relieved frequently by treatment of a variety of kinds, which indicate that in the case of eunuchoidism, especially the care given the subject incident to operation and treatment, may cause euphorea.[4] Undoubtedly, positive results have been obtained by transplanting ovaries from one subject into another and this is followed by sexual functioning, even to the extent of

[1] Dercum, F. X. (Emeritus Professor of Nervous and Mental Diseases, Jefferson Medical College). See The Biology of the Internal Secretions, Philadelphia, W. B. Saunders Co., 1924. Also Jour. Amer. Med. Assoc., vol. 84, p. 248, 1925.

[2] Greek *syn*, together, and *dromos*, running; that is, accompanying each other, being said of certain etiological features that appear when the disease develops.

[3] Greek *eune*, bed, and *echein*, to hold; bed-chamber attendants in the harems of the East, where they are known as eunuchs.

[4] Greek *eu*, well, and *pherein*, to bear; that is, having well-being, or "feeling well."

bringing forth offspring. Loeb[1] believes ovarian tissue controls sexual events and Doisy[2] has isolated a hormone[3] from the ovaries of rats; when this substance is injected[4] into castrated female rats,

Fig. 207.—Doisy's ovarian hormone. No. 805, control. No. 806, 1 mg. of the substance daily for four days. No. 807, 2 mgs. daily for four days. No. 808, control for No. 809. No. 809, 1 mg. daily for eight days. (Jour. Biol. Chem., vol. 61, p. 718, 1924.)

estrus occurs. The ovary, placenta, or liquor folliculi (but not embryo or corpora lutea) is extracted with alcohol, hydrolyzed by

[1] Loeb, Leo (Professor of Pathology, Washington University School of Medicine, St. Louis, Mo.), brother of Jacques Loeb (page 107). See Science, vol. 48, p. 273, 1918.

[2] Doisy, E. A. (Professor of Biochemistry, St. Louis University School of Medicine, St. Louis, Mo.). See page 24.

[3] Greek *'ormanein*, arouse to activity; see page 141.

[4] Doisy, in connection with collaborators (Allen, E. (Missouri), Pratt, J. P. (Ford Hosp., Detroit), see Jour. Amer. Med. Assoc., vol. 85, p. 399, 1925) reports that the effects of injecting the extract into spayed rats produced characteristic changes in the vaginal epithelium; these changes are utilized to define the ovarian hormone unit, which Doisy does as follows: One unit is the greatest dilution of extract of the ovarian follicle which, in three injections during a day, is followed by a characteristic vaginal epithelial change on the morning of the third day.

boiling with alkali, the sodium soap solution thus formed being evaporated to dryness and the hormone extracted by chloroform. Cholesterol, which is also extracted, is precipitated by digitonin,[1] leaving the hormone in solution. It is a heat-stable substance, soluble in fats and oils. The fact that corpora lutea do not contain this substance corroborates Loeb's theory in part, that corpus luteum controls ovulation.[2] In the male, the Steinach[3] operation consists of vasotomy (ligation and section of the vas deferens; removal of a portion of it near the tied part), thus supposedly causing the backing up of the secretion, especially of the prostate gland and of the sex cells, as distinct from the supporting interstitial cells of Leydig; or, by irradiation, the sex cells are destroyed. It is supposed that this operation or treatment permits the maintenance of a normal blood-supply to the interstitial cells, while the sex cells (spermatogonia and spermatozoa) are destroyed, and for this reason do not require nourishment; this permits a more abundant supply of materials for the interstitial cells. It is in the interstitial cells that a hormone is supposed to be produced. Perhaps psychic effects are encountered, which give the feeling of "rejuvenescence." It is known that operations similar to this, employed to sterilize criminals, give rise to prostate enlargement; this may cause the apparently beneficial effects. The evaluation of the Steinach operation, irradiation, thorium, or actinium treatment varies with different observers.

Premature development, or *heightened activity of the sex glands*, occurs generally associated with tumors of the sex glands, of the cortex of the adrenal, or with hypophyseal disturbances. It is not known whether administration of gland substance is favorable or not. However, surgical operation has led to astonishing results of curative value.

Osteomalacia[4] is a disease having some relation to the gonads. In it, the calcium metabolism is deranged. It is more common in women and is frequently accompanied by exophthalmic goiter and tetany. Castration generally retards the disease and may effect a cure. The condition is relieved by childbirth, or by induced abortion. Some favorable results have followed administration of pituitary substance. To attribute the disease definitely to disturbance of one endocrine system is not yet possible.

[1] A glucose from digitalis.

[2] See Corner, G. W. (Johns Hopkins University), Physiol. Revs., vol. 3, p. 457, 1923.

[3] Steinach, E. (University of Vienna, Austria).

[4] Greek *osteon*, bone, and *malakia*, softening.

Diseases Not Necessarily Connected with the Gonadal System. *Marie's disease*,[1] or acromegaly,[2] is attributed to the excessive activity of the anterior lobe of the pituitary gland. The nose, lips, tongue, jaw, feet, and hands become enlarged and also the sella turcica. The pituitary gland presses upon the brain and localization symptoms follow. The thyroid becomes affected, while other glands, like the sex glands, atrophy. Gland therapy has been of little aid, the chief form of treatment being either surgical removal of a portion of the gland, or Roentgen-ray application. Sometimes the disease is treated from the standpoint of the thyroid. The pituitary gland consists of anterior and posterior lobes, with the pars intermediary intercalated between them. The anterior lobe secretes one of the two hormones of the gland, namely, tethelin.[3] Pituitrin probably arises in the pars intermedia and is stored in the posterior lobe. We have already spoken of the secretion of the substance called pituitrin (page 663) and of Abel's preparation as a tartrate.[4] The disease diabetes insipidus, characterized by an excessive excretion of urine, seems to be correlated with lesions of the posterior lobe of the pituitary gland. Administration of pituitrin tartrate causes a reduction of urine excretion. The tartrate has pronounced pharmacological effects which are taken advantage of in obstetrics.

Fröhlich's[5] *Syndrome* is characterized by fatty deposition, or obesity, atrophy of the gonads and other glands of the endocrine system, lowered mental and physical activity of the body as a whole, optic involvement and lowered or suppressed action of the hypophysis. Treatment is by operation, or administration of gland substance, but in such gland feeding does not seem to affect the gland itself, but rather the body functions, such as the reduction of obesity. The sight, if impaired, is not benefited by gland therapy, because the growth of the gland infringes upon the optic nerves, and since the size of the gland is not reduced in therapy the optical defects are not bettered.

Gigantism is a condition best described as *early acromegaly*, although some observers (Biedl) believe the two to be different. The principal basis for differentiating the two diseases is that there is

[1] Marie, Pierre, French physician of the nineteenth century.

[2] Greek *akros*, top, and *megale*, great; meaning the extremities, which become enlarged in this disease.

[3] Tethelin, named by T. B. Robertson (Adelaide, South Australia). See his Biochemistry, Philadelphia, Lea & Febiger, 1924, 2d ed.

[4] Abel, J. J., Industrial and Eng. Chem., vol. 16, p. 1031, 1924.

[5] Fröhlich, A. (Austrian neurologist, contemporary). See Figs. 208 and 209.

a characteristic difference in the behavior of the gonads: In acromegaly there is a premature development, while in gigantism a condition similar to eunuchism is found. Many other glands belonging to the system of endocrines are affected in gigantism. The hypophysis may be involved, but hyperfunction of this organ is not alone the cause of gigantism. The adrenal cortex is enlarged, hyperplasia of the chromaffin system occurs, and enormous enlargement of the pancreas is characteristic. This last condition is commonly asso-

Fig. 208.—Fröhlich syndrome. Male. Service of Dr. Solis-Cohen, Jefferson Hospital. Note the deposition of fat, immature external genital organs, small stature. The sight is impaired, owing to the encroachment of the pituitary upon the optic nerves. (From S. Solis-Cohen and E. Weiss, Amer. Jour. Med. Sci., April, 1925; courtesy of Lea & Febiger.)

ciated with diabetes mellitus. The death rate of giants is high during the earlier years of life, due to the exhaustion of the secretions and the failure of the glands producing them. Gigantism has been cited as an example of the "pluriglandular diseases."

Multiple endocrine sclerosis is a second member of this group. It is characterized by deficiency in general, progressive weakness, anemia, failing mental vigor, defective senses, cachexia, and the development of conditions characteristic of special endocrine diseases, such as

Addison's disease, late eunuchoidism and adiposity. Brittleness of the nails, loosening of the teeth, and thinning of the bones are additional features. Therapy from the standpoint of endocrinology is uncertain, but particular gland substances, like thyroid, have beneficial results.

Pancreatic insufficiency, involving sugar and fat metabolism (diabetes mellitus), has been considered previously.[1] Insulin is the particular product in question. It is necessary for the utilization of

Fig. 209.—Sister of the subject of Fig. 208. (From S. Solis-Cohen and E. Weiss, Amer. Jour. Med. Sci., April, 1925; courtesy of Lea & Febiger.)

both sugar and fat. Administration of insulin restores, for a time, the normal function of glucid and lipid metabolism. We have spoken previously, also, of the beneficial use of whole gland substance along with insulin.[2] This gives some basis for the belief that insulin is not the only hormone of the pancreas, and that it is ineffective in the absence of that organ.

Diabetes insipidus[3] has been mentioned previously (page 667) as being characterized by abnormally high urinary output, with low or

[1] Pages 510 and 532. [2] Page 511.

[3] Greek *dic*, through, and *bainein*, to pass. The term is applied to any condition in which urine is abundantly excreted. Insipidus, from the Latin, meaning *without*

no sugar content, intense thirst, great appetite, loss of bodily vigor, adiposity, and sexual disturbances. There is no pathological histological finding indicating the cause of the disease, but it is known that polyuria, at least, is induced by irritating the hypophysis and the region surrounding this organ. The disease has been found associated with tumor of the hypophysis. It has been held in check by administration of pituitary substance. Feeding or injecting the posterior gland substance induced polyuria in normal subjects. The disease, then, concerns the posterior lobe and perhaps the pars intermedia and acromegaly the anterior portion of the gland.

General Considerations Concerning the Endocrines.—The criterion for the presence of an internal secretion depends upon: (1) The identification of the substance in the blood or lymph leaving the organ in which it is supposed to be produced.

(2) Symptoms which develop following excision of the organ producing the substance or suppression of its activity are affected by administration of the gland or substance isolated from it.

(3) Evidences of pathological symptoms appear after long periods of administration of the substance or gland from which it is procured.

The question remains, How many diseases mentioned above can be attributed to deficiency in or excessive production of endocrine substances? The reader will recall the relatively few instances in which the above criteria hold. Epinephrin has been detected in the blood leaving the glands, but in adrenal deficiency, administration of epinephrin is not of direct benefit. Again, administration of epinephrin over a period of any length does not produce conditions resembling those pathological states which are supposed to be due to hypersecretion of this substance. Turning to thyroxin, this substance has not been isolated from the efferent blood or lymph of the thyroid, but it is quite conceivable that the substance is given to the blood in such exceedingly small quantities that our means of analysis are inadequate. However, criteria (2) and (3) are readily demonstrated experimentally. In the case of insulin, all three criteria have been found applicable, and the chemically pure substance has been isolated (Fig. 213, page 709); its action can be demonstrated in the blood leaving

taste, and refers to the difference in taste between "sweet urine" (of diabetes mellitus) and the tasteless or salty urine of the patient with diabetes insipidus. The taste of the urine was for many years the chief means of differentiating these diseases, and in the rural districts, where physicians are unable to provide special laboratory facilities, the method is resorted to even at the present time.

the pancreas, thus signifying its presence; criteria (2) and (3) have been successfully applied. However, after leaving these substances, we have difficulty in assigning any of the three criteria to endocrine organs and the supposed secretions peculiar to them. There is no reason, however, why future work should not demonstrate the chemical nature of the substance derived from the pituitary, the gonads, and other organs. The work of Abel on pituitrin and of Collip on the parathyroid represents successful attempts.

The Interrelation of Organs of Internal Secretion.—These have been discussed by Hoskins[1] and by Carlson,[2] the latter warning that

Graphic Summary
Thyroid
Removal accelerates involution of thymus and prevents regeneration.
THYMUS
Gonads
Removal accelerates adrenal action. Gonads however are less active than adrenal in thymus effects.
Adrenal
Increases thymus activity; inhibits involution.

Chart showing the relations between the thymus gland and other endocrines.

"in this field appears the maximum fiction and the minimum of established facts." Carlson states that the most conspicuous correlations are as follows:

1. Dependence of the ovaries and testes on normal development and functioning of the thyroid and pituitary.

2. The interrelation between the hypophysis and the presence of normal thyroid and gonads.

[1] Hoskins, R. G. (Ohio State University; founder of the journal Endocrinology), Amer. Jour. Med. Sci., vol. 141, p. 154, 1911.

[2] Carlson, A. J. (Chicago), Jour. Amer. Med. Assoc., vol. 79, p. 98, 1922.

3. Thyroid administration over long periods induces adrenal and hypophyseal hyperplasia. Other organs are affected, making it impossible to believe that the effect is specific.

4. In experimental animals (larval frogs) excision of the thyroids leads to hypertrophy of the parathyroids. This has not been observed in mammals.

5. Lesions of the adrenal cortex (tumors) are correlated with premature sex development, vice versa, removal of the gonads has been observed to affect the cortex, causing hyperplasia.

6. Removal of the gonads exerts a retardation on the development of the thymus, but no other significant fact points to the affiliation of thymus with endocrine organs, or the identification of this organ as an organ of internal secretion. This view of Carlson may be somewhat modified by the recent statements of Marine,[1] who finds correlations between the thymus, adrenal, gonadal, and other glands of internal secretion.

The Rôle of Endocrine Organs in the Production of Disease. This has been discussed in the paper of Carlson just mentioned and by Dercum.[2] Let us review, briefly, the evidence submitted by Dercum:

Thymus.—This organ is associated with lymphatic hyperplasia and persistent thymus. Hyperplasia of the thyroid, pancreas, adrenal medulla, and of the testicles results from thymectomy. Thymic asthma and death are attributed to toxic conditions due to the interference in normal functioning of the thymus, since this organ is found to have undergone hyperplasia. However, mere traumatic pressure is inadequate to explain the conditions. The relation between persistent thymus and hypoplasia of the adrenal cortex has been asserted by several workers. Many cases of hyperthyroidism are accompanied by persistent thymus.

Thyroid.—The relation between thyroid and thymus has just been discussed. Hyperplasia of the one frequently accompanies hyperplasia of the other and the same may be said of hypoplastic conditions. Hyperthyroidism depresses pancreatic function, leading to glycosuria because of diminished insulin output. Connection exists between thyroid activity and the function of the sympathetic system, which may be correlated with the manifestations found in hyperthyroidism (flushing, etc.), in which it is probable that adrenal activity is stimu-

[1] Presented to the Federation of Societies of Experimental Biology and Medicine, Washington, 1924.

[2] Page 664.

lated by the thyroid by way of the sympathetic. Perhaps the exaggerated stature in hyperthyroidism is correlated with the hyperfunctioning of the pituitary, which is known to become enlarged, especially if hyperthyroidism begins during adolescence.

Pituitary.—As already indicated, the pituitary frequently enlarges in both hypo- and in hyperthyroidism, attributable, by Dercum, to compensatory development. In adipositas dolorosa the pituitary is affected, along with the thyroid, pointing to a common affection of the endocrine organs, since the sex glands (interstitial as well as primary sex cells) and adrenals are concerned. The thymus is abnormally persistent in many cases of Fröhlich syndrome and especially in the removal of the anterior lobe in experimental animals. The posterior lobe seems to have no correlative action with other endocrines, but secretes a special agent, similar in function to epinephrin.

Pancreas.—Hyperfunctioning of the pancreas is found in hyperthyroidism and in hyperpituitarism. Hyperfunctioning is observed in hypopituitarism. Endocrine disturbances leading to adiposity involve pancreatic hyperfunctioning. Thyroidectomy sometimes leads to pancreatic enlargement.

Adrenal Gland; Cortex.—Relations between the genital system and the adrenal cortex seem established. Hypertrophy of the cortex is associated with pseudohermaphroditism,[1] either congenital or acquired.

Adrenal Gland; Medulla.—This has already been referred to on pages 657 and 658.

Gonads: The sex glands are correlated with diminished thyroid, persistent thymus, and perhaps hyperpituitarism. The pineal bears some relation to the gonads.

Parathyroid.—Relations are supposed to exist between the parathyroids and the chromaffin system, the thymus and the thyroid.

Summary.—Carlson has summarized the findings from experimental data as follows: Extirpation of the following organs belonging to the endocrine system, or assumed to belong to it, leads to the results named:

Thyroid.—Cretinism (young); myxedema (adult). Induced myxedema never completely resembles the myxedema of the pathologist.

[1] Greek *pseudo*, false, *Hermes* (a god) and *Aphrodite* (a goddess), partaking of the properties of both sexes. In pseudohermaphroditism the condition is one in which while the primary sex cells remain either male or female, the bodily characteristics resemble the opposite sex.

Parathyroids.—Tetany; depression; death, unless especially controlled by diet or gland administration.[1]

Pancreas.—Diabetes mellitus.

Testes. Infantilism (young), loss of estrus, degeneration of the secondary sex characters.

Ovaries.—Infantilism; in the adult, premature menopause and atrophy of the secondary sex characters.

Adrenal Medulla.—No pathological state.

Adrenal Cortex.—Addison's disease, as judged by symptoms similar to those of human Addison's disease (prostration, etc.).

Pituitary, Anterior Lobe.—Infantilism (young); adiposity and loss of vigor (adult); atrophy of the gonads and loss of estrus; death (disputed by some).

Pituitary, Posterior Lobe.—No symptoms of pathological nature. Whether diabetes insipidus is produced by posterior lobe pituitarectomy or not is open for more critical work.

Pineal.—Without pathological results.

Thymus.—Without effect, unless adolescence is hastened.

As a general conclusion, Carlson believes that extirpation experiments afford no basis for believing that one endocrine system may substitute another.

Detoxication Function of the Endocrine System.—Experimentation has not yet revealed the answer to this question. It is not possible at present to state definitely whether organs of internal secretion function to care for the poisons which, under all conditions, are present in the body. We have discovered various chemical means for detoxicating substances. The many instances in which toxic symptoms develop following removal of one of the endocrine glands have led some observers to believe that the function of the gland is to inhibit intoxications.

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[1] Page 660.

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CHAPTER XV

THE EXCRETIONS OF THE BODY

"If you can measure that of which you speak, and can express it by a number, you know something; but if you cannot measure it, your knowledge is unsatisfactory."—*William Thomson (Lord Kelvin).*

We shall first consider various methods of studying the qualitative and quantitative chemistry of bodily excretions, as familiarity with this subject is essential to an understanding of the function of these substances in the organism, and to a formulation of a physiology of excretion. Most of the methods employed today in clinical laboratories have been devised by biochemical students of the Western Hemisphere, but, as a rule, European chemists contributed the fundamental principles. By these methods the excretory function of the urine, breath, perspiration, and feces have been determined. By them the clinician has been enabled to diagnose important diseases and to follow the course of the disease. The great discovery by Banting of insulin would be of little value in therapeutics, were it not possible to make an exact quantitative determination of blood-sugar and to govern administration thereby. This is accomplished by the method of Folin, Benedict, or others. The great importance of prognosis in modern medicine emphasizes the necessity of the student's familiarity with the methods and results of blood and urine analysis. For a practising physician and theoretical biochemist alike, the following pages are of the greatest importance.

Although the blood is an intermediary between the tissues and the excretory organs, and logically demands discussion at this point, the chemistry of the blood is better studied after the methods involved in urinalysis have been considered, for many of the blood chemistry methods are adaptations of those of urinalysis. A brief résumé of the methods of excretion of the products of metabolism follows:

Organic Substances:

1. *Products of glucid metabolism:*
   (*a*) Carbon dioxid is excreted in gaseous form in the breath, in conjugated form as constituents of the urine, perspiration, saliva, etc. Concerning the excretion of CO_2 as inorganic carbonate, see page 677 under Inorganic Substances.

(*b*) Water is excreted in the breath, urine, perspiration, and feces and chemically bound in many compounds.

(*c*) Intermediate products of glucid metabolism—acids and conjugated substances, like glucuronates—are excreted through the urine.

2. *Products of lipid metabolism:*

(*a*) As for glucids.

(*b*) As for glucids.

(*c*) Intermediary products of lipid metabolism, especially those formed in pathological states such as occur in acidosis, are excreted in the urine, although in severe cases the breath may contain certain products (aceton, in the acidosis of diabetes mellitus).

(*d*) Cholesterol and other sterols are excreted through the feces. The phospholipid phosphorus is excreted as inorganic phosphate in the urine. Recent investigations demonstrate that the phosphorus of the urinary "nucleoprotid" comes largely from exogenous sources.

3. *Products of protid metabolism:*

(*a*) As for glucids.

(*b*) As for glucids.

(*c*) Intermediate products of protid metabolism (peptids; amino-acids) are excreted by way of the urine.

(*d*) Aromatic rings belonging to amino-acids, like tyrosin, tryptophan, etc., are excreted as open-chain compounds, and are passed off in a manner similar to glucid products; or they are conjugated to form such products as hippuric acid. The latter is the usual way.

(*e*) The amino-group of protids are excreted largely through the urine as urea. Some nitrogen is excreted as purin, almost entirely through the urine, but to a small extent through the salivary and sweat glands. The nitrogen of the breath is derived from the air by inhalation, and, except for a small amount which may be mechanically retained for a short time, leaves the body on the next expiration.

Inorganic Substances.—These are principally salts, and are excreted in the urine, perspiration, and feces. Those in the urine are largely concerned with maintenance of proper acid-base relations in the body. Cystin sulphur and sulphur coming from other protid components is excreted as sulphate, inorganic and ethereal, but also

as unoxidized sulphur which escapes burning in the liver.[1] Phosphorus from the phospholipids, nucleoprotids, and elsewhere is excreted by way of the urine. Potential phosphoric and sulphuric acids in the form of salts, inorganic, conjugated, etc., are constantly constituents of the urine. Calcium is excreted through the urine, but largely through the feces. In the female, calcium is excreted also through the milk. Sodium chlorid is excreted through the urine, perspiration, alimentary glands, and feces. Iron is excreted through the feces.

THE URINE

Definition.—The urine[2] is a straw-colored liquid secreted by the kidneys, of aromatic odor and salty taste. Its average normal specific gravity is 1.015, and it gives the Jaffé reaction for creatinin.[3] Its constituents are derived from the blood as it passes through the kidney. Space does not permit a discussion of the various theories of the secretion of the urine, but the author inclines strongly to the essentials of the Ludwig hypothesis[4] of filtration. Richards[5] has shown that the flow of urine through the glomerulus varies with the blood-pressure, that the variations in concentration of urinary constituents run parallel with those in the blood, and other factors behave in such a manner that they may be reconciled with the theory mentioned above.

The kidney itself is not of great importance in the production of substances found in the urine, although several recent investigations have demonstrated that it is more actively engaged in syntheses[6] than was formerly believed.

Of the organic constituents, urea exists in the greatest amount, and is formed chiefly, if not entirely, in the liver. The ammonia of the urine is formed in the kidneys, and conjugated products, such as hippuric acid, are also produced in this organ, in part at least.

[1] Page 590.

[2] Greek *ouron*, derived from *reo*, to flow. The Latin is *urina*.

[3] Of the many identification tests for urine, the only reliable one is the demonstration of creatinin.

[4] For a statement of the theories see Brubaker's Physiology, cited on page 614, or Cushny.

[5] Richards, A. N., Professor of Pharmacology, University of Pennsylvania. See Richards, A. N., and Schmidt, C. F., Amer. Jour. Physiol., vol. 59, p. 489, 1922. Also Transactions of the College of Physicians, Philadelphia, volume for 1925.

[6] We refer to the formation of hippuric acid and of ammonia, both discussed in later sections (page 739 and Chapter XVI).

Inorganic substances found in the urine as, for example, sodium chlorid, are wholly derived from the blood. According to the modern conception of the formation of urine, we may say, that it is deprotidized blood, but the concentration of the various ingredients differs in the two cases, as some non-protid constituents of the blood do not occur in the urine except under pathological conditions. Thus, creatin is a normal constituent of human blood (0.0060 g. per cent.), but occurs in the urine in the adult male only in disease. Its appearance in the female is physiological after puberty, at sexual events like pregnancy and the menopause.

General Characteristics of the Urine.—The amount of urine excreted during a period of twenty-four hours may vary between wide limits, but the constituents are maintained fairly uniformly, other conditions being equal. This means that the amount of water in the urine is a changeable quantity. With the change in dilution of the urine, other properties change. The higher the water content of the urine, the lower the specific gravity, and the paler the color. The diet affects certain constituents; the more meat eaten, the greater the amount of water excreted, because of the diuretic effect of the urea produced from the meat and excreted in the urine. The more water excreted through the kidneys, the lower the specific gravity of the urine. Other urinary constituents are little affected by the diet. Creatinin is an example. The urine and the gastro-intestinal secretions are the two fluids of the body which depart radically from the neutrality of body fluids. Man, being an omnivore,[1] secretes a urine which is not as highly acid as that of carnivorous animals, but more acid than that of herbivores.

Clinical Value of Urine Studies.—The urine plays an important part in clinical diagnosis. Every examination for life insurance requires a qualitative, and frequently a quantitative, determination of its constituents. Each operation of any importance involves as a precautionary measure the examination of the urine. It is true that since the development of rapid and exact methods of blood analysis, urinalysis is of less importance in the diagnosis of many diseases, but both are often necessary for exact and detailed study. Urinalysis is frequently made in conjunction with blood analysis, as in the urea concentration tests of Ambard, MacLean, and others. From an examination of the urinary excretion over a given period, valuable data concerning the condition of the subject and his metabolism are obtained. The examination of the urine has led to an under-

[1] Latin *omne*, all, and *voro*, to eat; that is, capable of devouring a variety of foods.

standing of the normal and pathological behavior of foods, and we now know rather completely the history of foods as they undergo various changes in the body.

PHYSICAL CHARACTERISTICS

Volume of the Urine.—The average, normal, daily (twenty-four-hour) excretion of urine of adults in the United States amounts to about 1200 mls.[1] This figure may be modified in a given individual by one or more of the following conditions:

1. Air temperature, involving season of the year.
2. Characteristic constitutional state, whether phlegmatic or nervous. Racial characteristics are modifying causes.
3. Predominant character of the food, protid, fat, or starch. Use of coffee and beverages.
4. Amount of water intake as water, and in milk and beverages.
5. Daily variations of body temperature, reaction, etc.

1. *Temperature and the Season.*—During the warmer months, the skin excretes more water than during the winter months, and consequently the urinary volume is lower, owing to the diversion of water from the kidneys to the skin. For the same reason, urine is more concentrated in summer than in the colder months of the year. In the author's experience there is an average difference of one hundredth in the specific gravity of urine for summer and winter; that is, a summer urine 1.025, winter, 1.015.[2] Vasomotor reactions stimulated by changes in temperature constantly modify the amount of water excreted by the skin and by the kidneys. In a similar manner, but to a less extent, the water excreted by the lungs is correlated with that excreted through other organs, such as the kidneys and skin. Clothing has modified the sensitiveness of many persons to changes in temperature; owing to the mode of dress, the woman of civilized countries is more exposed to changes in temperature than men, so that she becomes inured to small changes.[3] The skin responds to

[1] It is true that the United States is subjected to great variations in climate and also that its population is a "melting-pot" of many races, but nevertheless there is enough homogeneity of conditions to make the average daily excretion of urine less than in the Teutonic countries of Europe, and somewhat more than in the Romance peoples of the Mediterranean districts. Germans who have come to the United States adopt a lower diet in protids. Dutch do much the same.

[2] It is customary in clinical biochemistry to drop the decimal point in speaking of the specific gravity, and henceforth we shall do so, but it must be understood that "ten-fifteen" means 1.015.

[3] During the exposure to cold winds and water, after the sinking of the Titanic by collision with an ice-berg, it was reported that women showed less effects of the rigorous conditions than the men.

changes in temperature somewhat according to the Weber-Fechner Law in psychology; before the skin responds to a gradual change in temperature, it is necessary that the temperature fall 3° C. A rapid fall in temperature is recognized even to the extent of half a degree, but such rapid changes are infrequently encountered by the unprotected skin of the civilized human being. An Eskimo may toil and perspire freely, sit down with his back to the wind and suffer no ill, while a similar behavior on the part of a tiro from civilization would probably lead to considerable discomfort.

2. *Race and Temperament.*—The amount of urine voided in twenty-four hours differs with race, probably owing to diet rather than any obscure cause. The Teutonic type generally excretes a larger volume of urine than the Romance type; but this difference may be correlated with the character of the diet, as the northern races eat much meat, which is conducive to increased excretion.

As a rule, individuals of excitable nature excrete more urine than those of a phlegmatic disposition. The mentally deranged, hysterical, and the insane void large volumes of urine. Normal persons, following unusual mental or emotional stress, have an increased flow of urine. This is found in students during examination periods. In some cases, however, such manifestations are caused by a large amount of beverages which contain diuretic substances (caffein in coffee, etc.).

3. *A diet high in protids* favors increased elimination of water in the urine. The reason for this is that the meat is digested to amino-acids which are absorbed and converted to urea, a well-known diuretic. This property of urea causes dilatation of the renal artery and other renal vessels, thus bringing more blood to the kidney. Coffee, tea, and chocolate act as diuretics, owing to the purin content mentioned above. Irritation of the urinary tract by medicines (cantharides; croton-oil, etc.), bacterial toxins, or perhaps by the bacteria themselves, tends to cause frequent micturition, which may or may not be a factor in increased fluid excretion.

4. *Intake of unusual amounts of water and milk* causes increased elimination of water, the excretion being about 400 mls. less per liter than the intake. There does not seem to be anything specific in these fluids. Whatever causes an increase in the volume of blood flowing through the kidney is conducive to an accelerated flow of urine.

5. *Day urine* is normally *greater in volume than night urine.*

	Mls.
Day urine	850
Night urine	350

Night urine greater in volume than 750 mls. indicates kidney trouble. The urine varies with the temperature of the body, which has a uniform variation throughout the day. The temperature (and urine volume) begins to rise about 6 A. M., regardless of such factors as exercise and diet, and this temperature elevation is accompanied by a sudden increase in urine excretion. The temperature reaches a peak about 4 P. M., which may be accompanied by a secondary increase in urine excretion. After 4 P. M. the temperature falls until morning, and there is an accompanying fall in urine excretion.[1] Under the heading Reaction more detailed study will be made of variations of urine during the day and night when correlated with hydrogen-ion concentration and other factors.[2]

Pathological variations in the volume of urine are found in the polyuria of diabetes mellitus and diabetes insipidus; in the polyuria accompanying lesions of the portions of the brain other than that associated with diabetes insipidus.[3] When water has been withheld in the tissues, as in nephritis, whether interstitial or parenchymatous, or in edema, pleurisy, etc., and then, owing to repair, there is a return to normal conditions, polyuria exists for a time. A decreased amount of urine is termed oliguria,[4] and this condition accompanies the low blood-pressure attendant upon heart disease, and is observed with fevers, diarrhea, and the toxemias of pregnancy. Total suppression of urine is known as anuria.[5] It occurs in bichlorid poisoning following a period of oliguria, in certain infectious diseases like scarlet fever, and during anesthesia. Anuria is not necessarily fatal, and may persist for over two weeks without an attending toxicity, called, uncritically, "uremia."[6] Brücke[7] has shown, by uniting the ureter with the common iliac vein, that the toxicity of urine arises when the urine remains in the ureter for some time; kidney urine is non-toxic.

[1] Simpson, G. E. (University of Pennsylvania), Jour. Biol. Chem., vol. 59, p. 107, 1924.

[2] For variations of urine constituents during morning hours see Folin's Laboratory Guide, 1925. [3] Page 667. [4] Greek *oligos*, small.

[5] Greek *a*, privitive (meaning without), and urine.

[6] Greek *ouron*, urine, and *'aima*, blood, or, literally, "urine in blood." For a method of detecting the presence of urine in blood see Tashiro, S., Medical Bull. Univ. Cincinnati, vol. 3, p. 8 1924.

[7] Brücke, E. Th. (Innsbruck) Über die Einleitung des Harns einer Niere ins Blut. xiith Intern. Physiol. Congress, Stockholm, 1926, Abstr., p. 28.

Summarizing:

Normal excretion of urine, from	800 to 2500 mls. per twenty-four hours.
Polyuria	2500 mls. or more.
Oliguria	800 mls. or less.
Anuria	total suppression of the urine.

Color of the Urine.— The color of urine may be described as yellow; the average normal urine is straw yellow. This color deepens when the urine becomes concentrated, as when too small an amount of water is ingested while the subject perspires freely. Departure from straw yellow is abnormal. Brownish or reddish urine indicates either disease or the presence of a food ingredient that affects the color. Rhubarb and cascara cause deep yellow urines, something like the canary yellow following santonin (see below). While departures from the straw yellow of normal urine are not necessarily pathological, they should arouse suspicion in the mind of the observer.

Variations in Normal Urine.—Pale yellow: Polyuria, due to any of the factors mentioned above; alkaline reaction, in some cases; temporary anemia.

Greenish yellow: Characteristic of night urines, and of polyuria in diabetes insipidus, in which case the color is very slight.

Canary yellow: This color is produced by the drug santonin, used to remove round-worms from the alimentary tract, especially in children. The drug paralyzes the smooth muscle-fibers of the worm, so that it cannot maintain its position in the tract, and if the host is given a purgative, such as calomel, the worm will be swept out with the feces.

Deep yellow: Concentrated urine, as mentioned above.

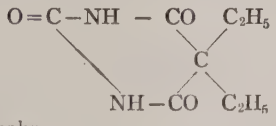
Amber yellow: Characteristic of day urines.

Pathological Variations.—Pale yellow: Diabetes mellitus;[1] pathological anemias,[2] chlorosis; renal lesions, such as interstitial nephritis.

Reddish yellow: Fevers; diseases of the lungs (pneumonia), liver (cirrhosis), etc. Bloody urines, as in children following use of turpentine for the cure of colds; in cases of lesions of the genito-urinary tract. Urea derivatives (veronal[3]), used as soporifics, produce hema-

[1] The pale yellow of this urine is correlated with high specific gravity which distinguishes it from diabetes insipidus and other conditions of pale yellow colored urines.

[2] But not pernicious anemia.

[3] Di-ethyl-barbituric acid,  Soporific, from the Latin *sopor*, sleep, and *facto*, to make.

toporphyrin,[1] which makes the urine reddish. This, in concentrated conditions, has a deep wine color.

Brownish-black urine: Icterus,[2] due to bile; concentrated blood-pigment, especially methemoglobin. Types of tumors (melanotic). Alkaptonuria;[3] extensive suppuration; intestinal stasis, as in cholera. Caution: Certain medicines cause urines almost identical in color with those produced by pathological states: creosote used in tuberculosis; resorcinol, used in checking diarrhea; also many coal-tar products.

Bluish-green urines: Pale greenish urines are voided by subjects with beginning icterus. A blue urine follows administration of the urinary antiseptic, methylene-blue; the colors in different stages after administration vary from green to blue and later become green again.

Taste of the urine, normally, is salty. In former times diabetes mellitus was diagnosed by the sweetish taste of the urine. The name of the disease was derived from this fact. (See page 669, note 3.)

Odor.—The urine has, under normal conditions, a characteristic odor, which is quite evident after considerable dilution. The odor is due to:

(1) "Urinoid," a compound of unknown chemical composition, which gives the characteristic odor of urine, even after all visible and chemical means of identification have been removed. In the preparation of urinary ingredients like creatinin (see below), the urinoid odor clings to the fractions after precipitation with picric acid and after extensive washings.

(2) Ammonia, which is normally present, but which increases on standing. The odor of the barnyard is largely that of ammonia.

The odor is modified by:

(1) Putrefactive changes in the bladder, as in urinary cystitis, the odor being aromatic, but different from the normal odor. Urine after being voided likewise undergoes a similar change. In cases of extensive pyelitis, a characteristic odor, a mixture of urinoid, ammonia, and hydrogen sulphide arises.

(2) The aceton odor, as found in acidosis, such as that of diabetes mellitus. In cases of anacidity, in which putrefactive changes occur, in the stomach and intestine, this odor appears.

(3) Fecal odor: Affections of the urinary tract as in perforations or rectovesical fistulæ from tuberculosis, malignancy, or radium, etc.

[1] Page 383.

[2] Greek *ikteros*, yellow. The disease is generally known as jaundice.

[3] Page 267. The color is due to oxidized homogentisic acid.

(4) Food and medicinal odors: Some food odors pass into the urine, as in the case of onions; asparagus gives rise to a sulphurous odor, due to methyl-mercaptan, CH_3SH. Turpentine and terpin hydrate give rise to an odor resembling that of violets. Menthol,[1] used to relieve pain, gives rise to a peppermint odor.

Appearance.—The appearance of urine, when viewed by transmitted light, is normally clear, but, even in health, this may be modified by the diet. The urine of herbivores, like the cow and horse, is turbid, owing to the precipitation of the urinary phosphates in the alkaline urine.[2] The urine of man behaves in a similar manner when the diet is vegetarian. Normal urine, on standing, develops a more or less distinct cloud, or nubecula,[3] which is due to the precipitation in acid urine of the mucin; the mucin is secreted by the lining of the urinary tract. Mixed with the mucus are pavement epithelial cells, and other cellular elements. Even acid urine, on standing, becomes alkaline through the action of bacteria on urea, which frees ammonia. Phosphates are precipitated under these circumstances. Bacteria in time will cause a cloud, since they multiply rapidly in standing urine to which no preservative has been applied.

Sediment.—Fresh urine should show no sediment, but after a meal rich in protids, a reddish sediment, due to urates, may appear when the urine has stood for a short time, or immediately after urination. Standing urine may show deposits of uric acid crystals and later of urates and of phosphates, like calcium phosphate. Sediment of calcium carbonate may appear. In certain diseases the sediment may contain erythrocytes, pus, casts of epithelium from the tubules of the kidney, and spermatozoa.

Reaction.—The mixed, twenty-four-hour urine of most persons is acid when freshly voided and tested with litmus-paper. Urine taken over short periods, however, may vary from actual alkalinity to its maximum acidity.[4] Following a meal there is a period known as the "alkaline tide" during which acidity becomes reduced and the urine neutral, or alkaline. The tide is most marked on a protid diet and least on a glucid meal. It is in direct relation to the amount of gas-

[1] Derived from a mixture of aromatic substances from the plant Mentha piperita or Hedoma sp. The mixture is called "oil-of-peppermint." Menthol (page 471) in the body gives the peculiar odor to the urine.

[2] For the cause of alkalinity in herbivorous urine see page 587.

[3] Latin *nubes*, cloud, and the suffix, *-cula*, diminutive.

[4] Urine cannot be made more acid than *p*H 5, nor more alkaline than *p*H 8. In the frog, at least, the urine of the uriniferous tubules is alkaline as far as evidence from injected dyes is concerned.

tric acidity due to hydrochloric acid, for the tide does not appear in anacidity and not usually after a period of fasting. Hubbard[1] believes that the secretion of gastric juice is the chief factor in determining the presence of an alkaline tide and its degree of development. The reason why the hydrochloric acid of the stomach determines the alkaline tide is that the acid-base balance of the body is affected

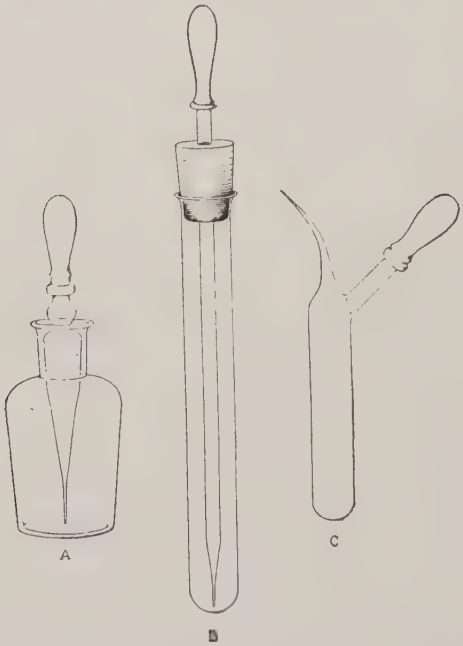


Fig. 210.—Felton's method for determination of pH in small amounts of fluid. Drop-plate method. A drop of the unknown is placed on a spot-plate and a drop of buffer containing an appropriate dye is added. This is continued until the colors match. (Jour. Biol. Chem., vol. 46, p. 302, 1921.)

by the secretion of HCl, and there are fewer acid radicles passing through the urine than during the period when HCl is not being excreted into the stomach. Folin[2] has shown that the acidity of the urine is due to (1) acid phosphates (BH_2PO_4), and (2) organic acids,[3] and the balance in such cases is determined by the total amount of acid radicles in the body. If the HCl drains away the H^+ (which deter-

[1] Hubbard, R. S. (p. 24). See Hubbard, R. S., Munford, S. A., and Allen, E. G., Gastric Secretion and the "Alkaline Tide" in Urine, Amer. Jour. Physiol., vol. 68, p. 207, 1924. Other references are given in this article.

[2] Folin, O., Amer. Jour. Physiol., vol. 13, p. 45, 1905.

[3] Lactic acid, for instance, occurs in normal amounts in urine at from 60–210 mgs. per liter of urine.

mines acidity), leaving the anion Cl^- to meet the cations Na^+, K^+, etc., a balance will be struck when there is a lowered excretion of acid-forming radicles in the urine. An obvious increase in the ammonia of the urine will result. It may be that the HCl is later absorbed by the walls of the intestine, into the blood, and there reduces the alkalinity during post-alkaline tide periods.

The relation between urine reaction and urine volume, mentioned above, is an inverse relation; during the night there is an increase in the acidity of the urine which is correlated with a decrease in the amount of urine secreted. The ammonia of the urine likewise increases.

The *degree of acidity of normal urine* may be expressed in terms of hydrogen-ion concentration, since true acidity, or effective reaction, is measured by the number of hydrions existing in the solution at the time. Normal human urine has an acidity thirty times[1] that of distilled water, or *p*H 5. If the reaction is determined as titratable acidity, then the true acidity is 0.0001 that obtained by titration. Titration, then, gives a false idea of the reaction of the urine, unless it is carefully controlled to avoid the dissociation of ammonia compounds and the precipitation of some of the acid phosphates of the urine. Folin[2] has devised such a method. The principle of the method is that a neutral solution of an oxalate (Na, or K) is added to a quantity of urine. The oxalate precipitates the calcium and thus prevents the formation of the insoluble tri-basic calcium phosphate when the solution becomes alkaline. Normally, the titration of KH_2PO_4 with KOH proceeds only as far as the removal of the second hydrogen:

$$KH_2PO_4 + KOH \longrightarrow K_2HPO_4 + H_2O.$$

When calcium is added, there is a liberation of hydrochloric acid, which augments the titration figure:

$$2K_2HPO_4 + 3CaCl_2 \longrightarrow 4KCl + 2HCl + Ca_3(PO_4)_2 \downarrow$$

As the presence of ammonium salts in the urine tends to make the end-point indefinite, because they hydrolyze with the liberation of free H^+ ions, it is necessary to use a large amount of indicator and to

[1] The *p*H of distilled water is 7; this means $(10)^{-7}$ or $\frac{1}{10\ 000\ 000}$ normal. Multiplied by 30, we have $\frac{1}{333\ 333+}$, or 3.3 $(10)^{-5}$, or about *p*H 4.7.

[2] Folin, O., Amer. Jour. Physiol., vol. 9, p. 265, 1903, and vol. 13, p. 102, 1905. See also his book, p. 165.

stop the titration at the first appearance of any increase in color. The titration should be carried out using a control, a beaker of the same amount of urine, potassium oxalate and the indicator.

Procedure: Pipette 20 mls. urine into a beaker and titrate, using 0.1 normal alkali:

(*a*) Directly.

(*b*) With the addition of 3 mls. neutral, saturated[1] potassium oxalate, KOOC.COOH.

(*c*) With the addition of 3 mls. neutral 2 per cent. calcium chlorid, $CaCl_2$.

(*d*) With the addition of 5 mls. each of the oxalate and calcium chlorid. Explain your results.

The result is expressed as the number of mls. of 0.1 normal alkali necessary to neutralize the acid in the full twenty-four-hour sample of urine. Normal urine gives about 600 mls. Estimated as hydrochloric acid, the titratable acidity in twenty-four hours is equal to approximately 2 gms. (36 drops) of the desk reagent.

Factors Modifying the Urinary Reaction.—(1) *Character of Food.* The reaction of urine runs parallel with the diet, an average vegetarian diet giving *p*H 6.6, whereas a high protid diet gives *p*H 5.9. Starvation, involving an almost exclusive protid metabolism, produces a urine of maximal acidity, *p*H 5. The phosphorus and sulphur of the protid foods are converted into potential phosphoric and sulphuric acids. The alkali metals, such as Na, from plant food yield carbonates. Different kinds of protid food cause different degrees of acidity, meat giving more acid than eggs, for example. A diet of fat tends to increase the urinary acidity, since the alkali radicles are used to produce soaps.

(2) *Time of Day.*—Urine voided during the day is less acid than that voided at night, perhaps owing to the lack of intake of food during the night, which throws the body virtually upon its own tissues for maintenance, with consequent similarity to the metabolism of carnivores. The difference between day and night urine has been attributed also to the differences in respiratory rate of these two periods, the tendency being for active respiratory movements to be correlated with a lessening of acidity, and lower respiration to increased acidity.[2]

[1] Thirty-two parts made up to 100 parts H_2O.

[2] Leathes, J. B. (page 203), Brit. Med. Jour., vol. for 1919, Part 2, p. 165, 1919; also Collip, J. B. (page 660), Jour. Biol. Chem., vol. 41, p. 473, 1920. The principle involved is evolution of CO_2 in active respiration.

(3) *Acid-base Equilibria in the Body.*—Of the potential acids formed during metabolism in the body, carbon dioxid passes off largely in the breath, while all other acid substances must be excreted in the urine. In general, there is a smaller intake of basic radicles in the food than of acid (page 588), and hence the equilibrium must be regulated to a certain extent by the kidney. This content of acid is increased under certain conditions, as in acidosis attendant upon exercise, as well as in pathological cases (see below). The alkali reserve is conserved by a mechanism in the kidney discovered by Benedict,[1] whereby ammonia is excreted to neutralize, as it may, acids which are brought to this organ in larger amounts than it can handle,[2] or perhaps even the usual amount of acid brought to the kidney by the blood. The ammonia replaces the blood bases and thus conserves them. The variation in reaction of the urine goes largely with that of the blood. Hence, factors of pathological nature, to be mentioned, affect first the reaction of the blood, and then that of the urine. As we shall see later, the alkali reserve may be estimated by determining the CO_2-combining power of the blood.[3] This power bears a numerical relation to the reaction of the urine expressed as the index of acid excretion:

$$\text{Index} = 80 - 5\sqrt[4]{\frac{D}{W}},$$

in which 80 is the maximum CO_2-combining power of blood; 5, the fourth root of the quantity (NH_3 — acid per liter of urine, expressed as 0.1 normal) and D the rate of this quantity excreted per twenty-four hours; W is the weight of the person in kilos. From 3 to 27 is normal; above 27 acidosis is indicated.[4]

(4) *Physiological States.*—At the beginning of exercise there is a decrease in the acidity of urine, due to the rapid loss of CO_2 through the lungs and consequent readjustment of the blood reaction. Later, and in the case of individuals inured to exercise, there is an increase in acidity due to acidosis, that is, the production of acids mainly from

[1] Benedict, S. R., and Nash, T. P., Jr. (Cornell Medical College, New York, N. Y.), Jour. Biol. Chem., vol. 48, p. 463, 1921; also Jour. Biol. Chem., vol. 51, p. 183, 1922.

[2] For further discussion of Benedict's work on ammonia in the blood, etc., see Chapter XVI. Benedict and Nash derived the ammonia either from urea or from amino-acids by deaminization.

[3] Page 76.

[4] When the reaction of the urine approaches the alkalinity of normal blood, the CO_2 combined in the plasma exceeds 71 mls. per 100 mls. plasma. (See page 71.) For references to this work see Fitz, R. and Van Slyke, D. D., J. Biol. Chem., vol. 30, page 389, 1917, and also; Barnett, G. D., J. Biol. Chem., vol. 33, page 267, 1918.

the metabolism of fat[1] which overburden the alkali reserve, and the ammonia formation which ordinarily compensates for increased acid production. Cold baths tend to produce urines of diminished acidity, and may cause alkaline urines. During exercise the lactic acid excretion of the urine increases from about 0.1 g. to as high as nearly 20 gs. per liter of urine.

Fasting causes an increased urinary acidity, since the person is virtually on a protid metabolism. The excretion of acid amounts to about 425 mls. expressed as 0.1 normal, per twenty four hours, when the excreted base is taken into consideration, or to about 925 mls. of total acid, if this factor is not considered. On an average diet the total acid excretion is about 950 mls., or, with bases subtracted, 120 mls. In the fetus, the total acid excretion is about 120 mls., but the bases excreted at the same time amount to 185 mls. giving a loss of base which must be met by the mother, and which probably accounts for the low alkali reserve of late pregnancy. In children up to one year of age, the excretion of acid varies according to whether the child is breast fed or is given cow's milk. Breast-fed infants excrete less acid than those fed on cow's milk:

Average acid excretion:	Mls. 0.1 normal acid.
Breast-fed infant	40
Cow-milk fed infant	250

For the child from one to ten years of age the acid excreted per twenty-four hours has been calculated at about 1834 mls., the value varying with the character of the food; fat, especially, causes an increase in acidity (page 688):

	Mls. 0.1 normal acid.
Excretion of acid on moderately fat diet	1670
Excretion of acid on high fat diet	1960

The distribution of acid excreted depends upon physiological states, but, on an average, it is as follows:

Acid excreted through the urine in twenty-four hours due to:	Mls. 0.1 normal.
Acid phosphate	100 to 200
Acid neutralized by NH_3	300 to 400
Acid as organic acid	0 to 150
Acid as carbonate	10 to 40

(5) *Pathological States.*—Acidosis is encountered in many diseases. Interference with blood-supply, interference with the oxidative

[1] Phosphoric acid is excreted in increased quantities after exercise, probably because of the breaking down of a hexosephosphate (p. 176) as glucose is used (as fructose).

powers of the body, and lowered oxygen tension in the air inhaled contribute to the production of acids in the body, the depletion of alkali reserve and overwhelming of the ammonia neutralizing power, with consequent excretion of acid. Since disease of any kind may cause reduction of gastric acidity, there is frequently a relative increase in acid excreted. In some diseases, however, the urine becomes alkaline. In anemia, in certain debilitating nervous diseases, in pneumonia when there is rapid absorption of the exudate, the urine becomes alkaline.[1] Alkaline urine is encountered in typhoid fever. In chronic interstitial nephritis, there may be a retention of phosphate in the blood, which causes a lower total acid excretion through the kidneys and a tendency towards alkaline reaction, or an actual alkalinity in the urine. A pseudo-alkalinity is encountered in urinary cystitis, or pyelitis, where bacteria cause the decomposition of urea in the urine while it remains in the bladder. Pseudo-alkalinity is easily distinguished from true metabolic alkalinity by testing the urine with red litmus-paper. True alkalinity turns the paper permanently blue, whereas the volatile alkalinity of decomposed urine causes the blue to appear, but the red color returns when the paper dries.

(6) *Artificial Alteration of Urine Reaction.*—In urinary cystitis and pyelitis the urine becomes alkaline, and permits the growth of bacteria which stimulate the formation of pus, and cause irritation of the bladder and urethral walls. It is necessary to change the reaction of the urine to acid. This is done by administering acid phosphate, NaH_2PO_4, which is normally responsible for the greater part of the acidity of the urine. Following this procedure, when the urine becomes acid, hexamethylene-tetramin[2] is given by mouth, and in the acidity of the urine the following reaction occurs:

$$\underset{\text{Urotropin.}}{(CH_2)_6N_4} + \underset{\text{Water.}}{6H_2O} = \underset{\text{Formaldehyde.}}{6H.CHO} + \underset{\text{Ammonia.}}{4NH_3}$$

The formaldehyde, thus freed, acts antiseptically. An alkaline, neutral, or low acid urine is desirable; in acidity, urinary calculi tend to develop. The alkaline reaction is acquired by (1) low protid, high vegetable and fruit diet, which increases the bases over the acid radicles; (2) medicinal administration in the form of sodium bicar-

[1] This does not indicate a fault in the acid-base metabolism, for investigators in Van Slyke's department at the Rockefeller Hospital have shown that the blood alkali reserve is practically normal. (Hastings, A. B., Neill, J. M., Morgan, H. J., and Binger, C. A. L., Proc. Soc. Exp. Biol. and Med., vol. 21, p. 66, 1923.)

[2] Commercially known as "urotropin" (Greek *ouron*, urine, and *trope*, food).

bonate, citrates,[1] etc. Bases must be furnished to pregnant women and to children. This is the basis of the value of moderate protid and liberal fruit and vegetable diets for such persons. On the other hand, acid diets are indicated in certain diseases, as in infantile tetany and rickets.[2]

Specific Gravity.[3]—The specific gravity of the urine varies widely under physiological and pathological states. In general, urinary volume and specific gravity are inversely proportionate; when the volume is high, the specific gravity is low. This, however, is by no means invariably the case, and the exceptions are of value in clinical diagnosis. In diabetes mellitus the volume and specific gravity are both elevated, owing to polyuria and to glucose and accompanying substances.

There are the same seasonal and environmental variations, as in the case of urinary volume. In summer, the specific gravity increases, especially when there is not enough liquid in the diet. The average normal specific gravity is 1.015. The variations are as follows:

Seasonal.—These have been discussed on page 680.

Hourly.—Considerable variation between day and night urine, and urine excreted from hour to hour, is characteristic of a normal state. "Fixation of specific gravity," that is, flattening out of the curve of urine excretion, indicating a lessening of the variation in specific gravity from period to period, is pathognomonic. This is especially true if the lessening occurs while the specific gravity is low, that is, 1015 or below. A difference of from 8 to 9 points in the specific gravity for two hour periods is normal for day urines. The specific gravities of day and night urines normally are not widely different, but there must be slight differences in the concentration

[1] Citrates become oxidized to carbonates, which explains the anomaly that "acid" fruits, like lemons, furnish base; page 588.

[2] Zucker, T. F., Johnson, W. C., and Barnett, M. (Columbia University, New York, N. Y.), Proc. Soc. Exp. Biol. and Med., vol. 20, p. 20, 1922. Also see Greenwald, I. (Harriman Research Laboratory, Roosevelt Hospital, New York City, N. Y.), Endocrinology and Metabolism, edited by L. F. Barker, New York, D. Appleton & Co., 1922, vol. 3.

[3] This term may be defined as the ratio between the weight of a unit amount of a substance like urine, etc., and the weight of a similar amount of pure water at its greatest density, 4° C. Unless the measurements are made at the same place on the earth's surface, the ratio must be made between the masses of the liquids, since weight varies with latitude and altitude. For example, the specific gravity of sulphuric acid at its highest purity as sold on the market is 1.84, which means that 1 ml. weighs 1.84 g. when pure water weighs 1 g. per ml. Similarly, a liter of pure sulphuric acid weighs 1840 gs. when a liter of pure water weighs 1000 gs.

of day urine to compensate for variations in environmental conditions, whereas at night these conditions are usually constant.

Pathological Variations.—Some of these have been indicated above. After taking a liter and a half of water, the normal kidney should produce a specific gravity of very low order (1005 or below). At the end of a day without much liquid, the specific gravity should be about 1030 or above. Loss of this power has been utilized as a means of detecting renal disease. The reader will refer for further details of pathological variation to the discussion of variations in volume on page 682. For a consideration of the characteristic variations in the urine see the writings of Mosenthal.[1]

The *method of estimating specific gravity* of the urine in common use is by means of a hydrometer—a glass bulb with an elongated

Fig. 211.—Urinometer for determining the specific gravity of urine. The hydrometer is first tested with distilled water to insure that the unit point is accurate. The readings are then taken on the urine and expressed clinically as "ten-twelve," "ten-thirty," equivalent to 1.012 and to 1.030, respectively.

stem. The bulb is weighted with mercury, or with shot, and the stem is graduated in degrees on the Gay-Lussac scale, distilled water at 4° C. being unity. The bulb is dropped into the urine and permitted to settle; then the graduation opposite the meniscus of the surface of the urine is read. Few of the commercial instruments are accurate, and all should be standardized against distilled water at the temperature indicated on the bulb. Some firms standardize at 15° C. and others at 20° C. A plus or minus factor must be carried if the instrument deviates from the volume at the standard temperature with water. The most accurate method for determining specific grav-

[1] Mosenthal, H. O. (Columbia University and New York Post-Graduate Medical School), Renal Function as Measured by the Elimination of Fluids, etc., Arch. Int. Med., vol. 16, p. 733, 1915.

ity is by means of a pyknometer, or specific gravity bottle or pipette. The Ostwald type is readily used. It is first filled with distilled water to the mark and weighed, then emptied and filled with urine. This sample is discarded and the pyknometer again filled with urine, thus insuring an undiluted sample for estimation. The instrument is weighed. Finally, after emptying, it is rinsed with pure water and dried by passing a current of air through it, after which it is weighed for the third time. Then:

$$\text{Specific gravity} = \frac{\text{Weight of tube + urine − wt. dry}}{\text{Wt. of tube + } H_2O \text{ − wt. dry.}}$$

Total Solids.—In general, the total solids[1] of the urine are correlated with the specific gravity, since the constituents of the urine vary but slightly qualitatively, although they may vary considerably quantitatively. Various factors have been suggested which, when multiplied by the last two figures of the specific gravity (those of the second and third places of decimals), give approximately the number of grams of solids left per liter of urine when the volatile portions of the urine have been evaporated.

Haeser's coefficient: Multiply the last two figures by 2.33.
Long's coefficient: Multiply the last two figures by 2.66.

These results are expressed in terms of liters of urine.

Haines' coefficient: Multiply the last two figures by 1.10.

This result gives the grains (60 mgs.) of solids per ounce (Troy wt., 31.1 gs.). Long's[2] coefficient is the one which seems best to approximate the total solids in the urine of the average subject in the United States.

The viscosity of the urine bears a relation to the specific gravity and to volume, according to the formula: Visc. = (sp. gr. − 1.001) 2200, in which water is unity and the specific gravity expressed in parts per thousand. Viscosity varies inversely as the volume.[3]

The surface tension of urine is about 60 dynes per centimeter (water 77).

[1] By this term is meant the non-volatile material of the urine, expressed as grams per unit volume.

[2] Long, J. H. (deceased), formerly Professor of Chemistry, Northwestern Medical School, Chicago, Illinois.

[3] Hahn, F.-V. von. Kolloidchemische Harnuntersuchungen. Die Beziehung der Viskosität des normalen Harns zum spezifischen Gewicht, zur Harnmenge, usn. Biochem. Zeitschr., vol. 178, page 282, 1926.

Chemical Characteristics of the Urine

Nitrogen Elimination.—Nitrogenous substances may be excreted through any avenue of excretion, but metabolic nitrogen is ordinarily voided through the urine. We have referred in another Chapter[1] to the fact that in any consideration of the balance between intake and output of nitrogen, it is necessary to consider only urinary nitrogen, for the Munich school of investigators[2] found that nitrogen which does not pass through the urine is negligible. A small amount of urea is excreted through the perspiration; a smaller amount of uric acid, from the salivary glands into the mouth; and a small amount of ammonia, through the breath; but taken together, these extra-urinary nitrogenous substances amount to little compared to those excreted through the urine. The feces contain nitrogen, but this nitrogen is largely derived from bacteria, from secretions of the alimentary tract, and from food which has not been absorbed.

Carbon, Hydrogen, and Oxygen Elimination through the Kidney. While no other excretory system, neither skin, lung, nor intestine, shares with the kidney the function of excretion of nitrogen to any extent, the *urine* is but *one of several* systems for the excretion of substances composed of carbon, hydrogen, and oxygen. The skin, the lungs, and the intestine excrete water. The kidney, likewise, shares in this process. The lungs excrete carbon dioxid. The kidney shares in this function, the carbon dioxid being linked chemically with ammonia in urea[3] and in other substances about to be discussed.

Inorganic Substances.—The kidney excretes a variety of inorganic substances, such as phosphates and carbonates. This function seems to be the oldest of all the powers of the renal apparatus, since it chiefly involves the maintenance of proper osmotic pressure relations. The primitive organism from which the higher forms of animals have developed was segmented into a series of compartments, from the anterior to the posterior end of the body. Such an organism lived in the sea where the osmotic pressure was considerable, and it was necessary that an efficient regulatory mechanism be provided to keep the osmotic pressure of the organism similar to that of the surrounding medium; otherwise, the compartments would collapse or expand. Each compartment communicated with the outside by means of a pair of cœlomo-ducts through which salts and water could be excreted to maintain the proper balance. Modifications of these

[1] Page 618.

[2] Pages 24 and 595.

[3] Or, more properly, what would become CO_2 unless urea were formed. See page 80 and page 539 concerning urea synthesis.

ducts have occurred, but they are recognized by the morphologist in the present-day mammal. Probably the exchange of a new medium, air, for the aqueous medium of former times, caused considerable change in the excretory system. Moreover, some parts of the system were appropriated by the genital apparatus as a means of emitting its products, eggs and spermatozoa. The excretion of nitrogenous substances may be considered a secondary process for the kidney, and doubtless one which was foisted upon it in a manner similar to that of the expulsion of sex products.

Organic Excretory Products: Nitrogenous.—Total elimination of nitrogenous products; "total nitrogen." In twenty-four hours the average adult human male excretes through the urine about 15 gs. of nitrogen. This nitrogen is not free, but is contained in a variety of substances, particularly urea, uric acid, ammonia, creatinin, and hippuric acid. The nitrogen contained in each of these substances bears a more or less definite proportion to the total amount of nitrogen excreted. Thus, with urea,

$$\frac{\text{Urea nitrogen}}{\text{Total nitrogen}} = \frac{85}{100} = 0.85, \text{ or } 85 \text{ per cent.}$$

On a total excretion of 15 gs. of nitrogen, 85 per cent., or 12.8 gs. of urea may be expected as normal. This total nitrogen may be altered by the diet, by physiological states and by pathological conditions. It rises on a high protid diet, and falls on a low protid or liberal glucid diet. It rises with an accelerated basal metabolic rate, falls with a subnormal one. It falls in cases of renal involvement (retention). The average total nitrogen excretion in warm climate, such as the Southern States, has been studied by Denis. She finds for Tulane[1] students 10.6 gs. per twenty-four hours. The corresponding figures for the Northern States are from 15 to 18 grams. Other conditions modifying the excretion of total nitrogen, and of certain nitrogenous substances, will be mentioned in the course of the following discussion.

The *method of measuring total urinary nitrogen*, whatever may be the modification, primarily depends upon the following principles:

1. Conversion of the nitrogen to ammonium sulphate by boiling the sample with substances containing the radicle $SO_4^=$. Sulphuric acid, cupric sulphate, and potassium sulphate are usually used.

2. Decomposition of the ammonium sulphate formed in (1) by alkali, thus freeing NH_3.

[1] Dr. W. Denis is Professor of Biochemistry at Tulane University Medical School, New Orleans, La.

3. Determination of the ammonia by:

(*a*) Kjeldahl's method: Aspirating the NH_3 into known amount of standard acid and titrating excess acid left after all the ammonia has been neutralized (page 287).

(*b*) Folin's method: Aspirating as before, determining the ammonia content by treating the fluid with Nessler's Solution, and comparing the color thus obtained with a standard color corresponding to a known ammonia content.

The Kjeldahl and Folin methods have many variations, some of which will be given later.[1] In the case of pathological urines containing protid, as in the forms of Bright's disease, the albumin must be removed before the determination is made; for total urinary nitrogen means non-protid nitrogen.

Kjeldahl Method for Total Urinary Nitrogen.—This method has been outlined on page 287, in which casein was used as the substance to be analyzed. Using urine, pipette 5 mls. of the protid-free filtrate (if albumin is present), or simply 5 mls. of the urine, if protid free, into a 500- to 800-ml. Kjeldahl flask (Fig. 108, page 288) and follow the procedure for the method given on that page.

Folin's Method for Total Urinary Nitrogen; Macroform.—Principle: The principle is the same as that of the Kjeldahl method.

Procedure: Transfer 5 mls. of urine to a Kjeldahl flask. Add 5 mls. of the special (Appendix) phosphoric-sulphuric acid mixture, also 2 mls. of 10 per cent. ferric chloride solution, and small pebbles to prevent "bumping." Boil vigorously over a microburner. In three to four minutes the foam which forms at first will have entirely disappeared and the flask filled with dense white fumes. When this stage is reached (but no earlier) cover the mouth of the flask with a crucible cover and continue heating for two minutes. Then dilute urines, will be greenish, concentrated urines, a light yellow; the black matter will be completely destroyed. The flame should then be turned very low and the gently boiling process should be continued for two minutes, making a total boiling period of four minutes, counting from the time the cover was put in place. Remove the flame and let the flask cool for four to five minutes. At the end of four or not more than five minutes add, *first*, 50 mls. of water, then 15 mls. of saturated sodium hydroxide (50 per cent. solution) and connect the flask promptly, by means of a rubber stopper and ordinary glass tubing, with a 300-ml. flask for receiver. In the flask place 75 mls. of 0.1 normal acid, about 150 mls. of distilled water, and a

[1] Pages 697 and 698 and Chapter XVI.

drop or two of indicator (alizarin, etc.). As soon as the connection is made apply the microburner flame. Boil five minutes. Disconnect, remove the flame, and titrate the contents of the receiving flask against decinormal alkali as in the Kjeldahl method. The calculation is the same as in that method.

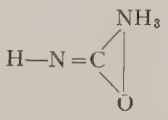
Folin's Method; Microform.—Principle: Colorimetric comparison of the ammonia derived from a Kjeldahl-like procedure (but without aspirating or distilling into normal acid), with the color developed by a known concentration of nitrogen as ammonium sulphate. Procedure: Dilute the urine according to its content of nitrogen: Normal urine, 10 mls. up to 100 mls. with distilled water; urines low in nitrogen, 20 mls. up to 100 mls.; those high in nitrogen, 5 mls. up to 100 mls. The optimal amount of nitrogen is about 1 mg. nitrogen in the amount of urine being analyzed. Of the diluted urine, well mixed, pipette 1 ml. (Ostwald-Folin pipette) into a 200 × 20 mm. Pyrex test-tube and add 1 ml. of the special acid mixture (Appendix) and a piece of Pyrex or quartz pebble to prevent "bumping." Hold the tube by means of a test-tube holder (or clamp it in a burette clamp) and by means of a microburner make the contents boil until the whitish vapors referred to above appear. Then cover the mouth of the tube with an inverted crucible cover. Continue heating until the mass in the bottom of the tube turns brownish, then black, and then, finally, clear yellowish green or (preferably) green. Continue boiling one minute longer. The total period must be not less than two minutes. Remove the flame, let cool spontaneously for (preferably) 90 seconds, and then cautiously add distilled water from your wash-bottle until about 10 mls. have been added. Transfer the fluid to a 200-ml.[1] volumetric flask. Add about 10 mls. more water to the tube, as wash-water and combine the washings, repeating until you have a volume of about 150 mls. Preparation of the standard: Pipette into a similar 200-ml. volumetric flask, 10 mls. of the standard $(NH_4)_2SO_4$ solution, thus adding 1 mg. nitrogen to the standard flask. Add 1 ml. acid mixture and water to make about 150 mls. of volume. Then rotate the flasks and add, as nearly at the same time as possible, 30 mls. of Nessler's solution (Appendix). Agitate the contents and dilute to the mark. Mix. Compare in a colorimeter and apply Beer's formula (page 170). For more critical work Benedict (page 31) recommends two modifications: (1) Distillation as in the Kjeldahl method (modified) and (2) direct method involving

[1] In this and similar instances, a 250 ml. volumetric flask may be used, but a similar flask must be used for the standard.

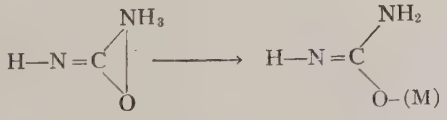
(*a*) Dilution of the urine so that the analyzed specimen contains about 5 mgs. nitrogen, that is, about five times the amount used by Folin; (*b*) boiling as in the Folin method, but omitting the cupric sulphate from the acid mixture, and (*c*) diluting to 50 mls. (in a 50-ml. volumetric flask). Of this 50-ml. solution 10 mls. are pipetted into a 100-ml. volumetric flask. Direct Nesslerization is then performed as in the Folin procedure by adding distilled water to make about 50 mls. of fluid, then 30 mls. Nessler's solution, and finally water to make up to the mark, 100 mls. The colorimetric comparison and calculation are the same as in the Folin method.

Nitrogen Partition.—For practically all purposes, whether research or clinical diagnosis, it is desirable to distribute the total nitrogen among the components already mentioned, urea, uric acid, etc. In order to do this it is necessary to determine the urea nitrogen. uric acid nitrogen, etc. Before this can be done intelligently the chief chemical characteristics of these various substances must be known.

Urea occurs in normal urine in amounts of about 10 gs. as urea nitrogen, or 30 gs. as dry urea in twenty-four hours. In three hours, during the morning, about 1.12 g. is excreted as urea nitrogen. Chemical definition: Urea is an ammonio-keto-cyanate:



The cyclic formula is demanded by the chemical characteristics of dry urea, among which is the relatively stable nature of the compound. Salts of urea are found in the urine, the cyclic ring being opened in the presence of water.



Urea (dry). "Iso-urea" (combining form).

Throughout this book urea is considered as a cyclic cyanic compound, following the investigations of Woehler, who first artificially synthesized it, and who found that urea is formed "par l'action du cyanogène sur l'ammoniaque liquide;" the investigations of the physical-chemist Walker and collaborators, who showed that "Woehler's synthesis" is a bimolecular reaction and that the reaction is reversible, which means that this synthesis is not a mere isomeric trans-

formation of ammonium cyanate to ammonium carbamate (urea), but must consist of a double reaction; the investigations of Gamgee, who demonstrated that the two atoms of nitrogen are not connected up in the molecule of urea in the same manner, and also that urea is more refractory as a compound than the usual "carbamid" formula, $C{=}O{<}^{NH_2}_{NH_2}$ would lead one to believe; the investigations of Heinze and others to the effect that one of the nitrogens of urea is pentavalent; the necessity of making a formula for urea different from the usual carbamid formula, to accommodate the salts of urea; the conclusions of Werner following his extensive studies[1] of cyanogen compounds, carbamates, urea, and allied substances. Urea crystallizes in long, white, tetragonal prism crystals which have a salty taste. They are readily soluble in cold and hot water; in all strengths of ethanol[2] up to 20 per cent., and in aceton. Urea is slightly soluble in ether and in chloroform. The crystals melt at 132° C., and at their boiling-point they undergo decomposition, which takes place at about 180° C. The following table from Werner shows the effect of heating urea:

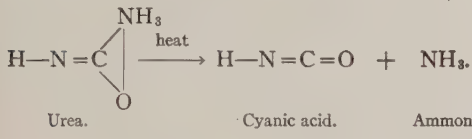
At about 180° C. Time of heating: twelve minutes.	Percentage.	At about 195° C. Time: six to seven minutes, rapidly.
Volatilized and lost as NH_3 and HNCO...	8.40	19.00
Ammonium cyanate, $NH_4.OCN$, sublimed.	0.58	0.42
Biuret, $HN(CONH_2)_2$.....	18.26	10.25
Cyanuric acid, $C_3H_3N_3O_3.2H_2O$.....	3.35	15.34
Cyanuromonamid, or ammelide, $C_3H_4N_4O_2$	0.83	8.46
Urea unchanged after twelve minutes.....	68.58	46.53

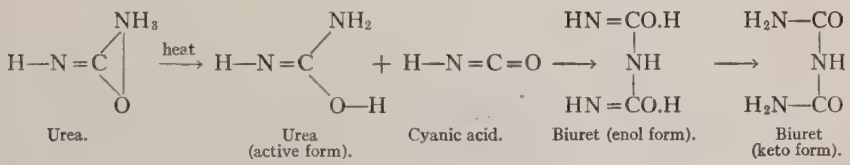
Tests for Urea: As Dry Urea.—Transfer a "knife-point" of the dry material to a long, narrow test-tube, preferably of hard glass. Heat the lower part of the tube over a low microburner flame, but allow the upper part to remain cool and act as a condenser. Test the fumes with red litmus-paper as the substance fuses; in the presence of decomposed urea the paper turns blue, indicating the presence of ammonia. Now permit a few drops of cobalt nitrate solution to pass down the sides of the tube; as the drops come in contact with the sublimed substance a blue color develops, indicating the presence of cyanic acid, HNCO. Again, pass a test-tube

[1] For a complete discussion see his book.

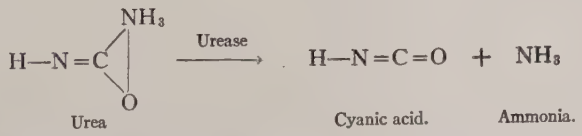
[2] At 70° C., 100 mls. of ethanol 98.6 per cent. strength dissolve 17.5 gs. urea.

sponge cleaner down the tube past the sublimate and wash the sponge in about 1 ml. of distilled water in a small evaporating dish. To the liquid in the dish add two small crystals of hydroxylamin hydrochlorid, $H_2N.OH.HCl$, and let them dissolve, after which add ferric chlorid, 5 per cent. solution, until a change of color appears. In the presence of cyanic acid a purplish-blue color develops.[1] Lastly, to the substance remaining at the bottom of the test-tube add 2 mls. of 10 per cent. NaOH solution and a drop of very dilute cupric sulphate solution; a pinkish color appears, due to the presence of biuret, $HN(CONH_2)_2$. The reactions involved in the foregoing procedures are as follows:





Urea in Solution.—(*a*) By Urease: To 5 mls. of the solution in a test-tube add 5 drops of the indicator phenolsulphonephthaleïn. By means of a stirring-rod transfer drops of acetic acid (1:200 solution) to the test-tube until the color is a yellowish pink,[2] which is slightly acid. Add a generous "knife-point" of jack-bean meal to the tube, shake to mix the meal and the fluid thoroughly, and place the tube in a water-bath at 50° C. At the end of five minutes examine the preparation; if no color change has occurred, leave another five minutes or, at most, fifteen minutes. The presence of urea is indicated by the purplish-red color of the alkaline solution of the indicator, due to the ammonia which has been dissociated from the urea under the dissociating action of the enzyme, urease, of the meal as follows:



[1] If less than about a gram of urea is taken in these tests, the sublimate should be scraped from the broken tube and transferred to the evaporating dish.

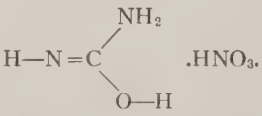
[2] The color should be checked with the standards.

The cyanic acid then undergoes hydrolysis as follows:

$$\underset{\text{Cyanic acid}}{H{-}N{=}C{=}O} + \underset{\text{Water}}{H_2O} = \underset{\text{Ammonia.}}{NH_3} + \underset{\text{Carbon dioxid.}}{CO_2}$$

Ammonium cyanate, biuret, and other combinations occur, due to interactions. This enzyme reaction is employed in the quantitative determination of urea in blood, urine, etc. (page 824).[1]

(*b*) By Precipitation as Urea Nitrate: Place 5 mls. of freshly voided urine in a test-tube and dilute[2] with one volume (5 mls.) of distilled water. Mix and transfer 1 or 2 drops of the solution to a microscope slide; add a drop of cold, concentrated colorless[3] nitric acid. Cover with a cover-glass and examine under the microscope for crystals of urea nitrate.



(*c*) By Precipitation as Dixanthylurea:[4] To 2 mls. of fresh urine in a test-tube, add 7 mls. glacial acetic acid; mix. Add to the solution 1 ml. 10 per cent. solution xanthydrol in methanol.[5] Let the preparation stand for five minutes or longer until crystals apppear. Examine a drop of the crystals suspended in the solution under the lower power of the microscope. The crystals are in the form of fine, radiating filaments. Filter the remaining solution through a dry paper marked with your initials, lay the paper upon a watch-glass and place in the oven. When dry note the delicate reddish or pinkish color of the crystals.

Preparation of Urea from Urine.—This is done readily by following the principles of the urea nitrate procedure given above.

[1] Although Sumner (page 24) was unable to detect cyanic acid in a urea-urease preparation, Fearon (page 710) has succeeded in demonstrating the presence by means of a special color test. (See Fearon, W. R. Journ. Biol. Chem., vol. 70, p. 785, 1926.)

[2] The purpose of this dilution is to obviate the retarding effect on crystallization of the substances in the urine of colloidal nature.

[3] Nitric acid containing nitrous acid decomposes urea. See page 294.

[4] Fosse, R. (French chemist), Sur l'identification de l'urée, et cetera. Comptes rendus (Paris), vol. 157, p. 948, 1913.

[5] Xanthydrol is made from xanthone, which, in turn, can be obtained by heating salol, $HO.C_6H_4.CO_2.C_6H_5$, when it is converted into the diphenylene keto-oxid, or xanthone. Xanthone is converted into xanthydrol by reducing it with zinc. For the method of preparing xanthydrol from xanthone see Fosse, R., Annal. Chimie, vol. 6, p. 13, and p. 155, 1916. The chemically pure xanthydrol is obtainable from the A. H. Thomas Co., West Washington Square, Philadelphia, Penna.

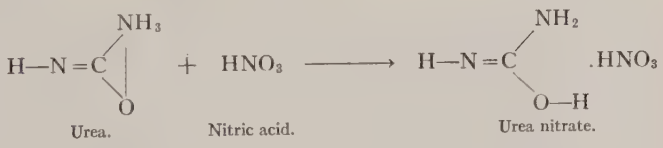
Filter 500 mls. of fresh urine[1] into a large evaporating dish and boil over a moderate flame until its volume is about 100 mls. Pour this liquid into an Erlenmeyer flask of 500 mls. capacity and stand the vessel in chopped ice-salt mixture. When the temperature has fallen to 0° C., add 100 mls. of nitric acid,[2] which has been standing in the ice-salt mixture until this time. Mix and after about fifteen minutes examine for crystals. Filter, but do not wash with water. Scrape the crystals from the paper into a large evaporating dish and add barium carbonate, creamy, aqueous solution, until the liquid no longer effervesces. Place the dish on a steam-bath, evaporate to dryness,[3] and extract the residue with 50 mls. of ethanol at 50° C., the alcohol being heated on the water-bath and care taken to avoid ignition. Repeat the extraction once with a similar amount of ethanol and combine the extracts in an Erlenmeyer flask. Add 5 gs. of animal charcoal, mix and filter, repeating if necessary until a clear solution is obtained. Concentrate the filtrate to a thin syrup on the water-bath and permit to cool. Crystals of practically pure urea are obtained.

The Production of Molecular Nitrogen by the Decomposition of Urea.—This process involves two important reactions:

1. The action of nitrous acid upon urea.
2. The action of hypohalogenites upon urea.

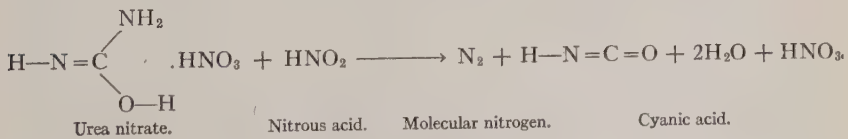
1. The Nitrous Acid Reaction: The equations are as follows:

(*a*) The production of an additive compound of urea by the action of a strong acid, like nitric acid:



Urea. Nitric acid. Urea nitrate.

(*b*) The production of molecular nitrogen and by-products:



Urea nitrate. Nitrous acid. Molecular nitrogen. Cyanic acid.

[1] See page 869 for the method of collecting and preserving urine for practical work.

[2] For the kind of acid see page 702, note 3.

[3] The preparation may be left on the bath until the following period, properly labelled.

Cyanic acid is hydrolyzed, either:

A. By means of strong acid: $H—N=C=O + 2H_2O \xrightarrow{HNO_3} NH_4OH + CO_2$.

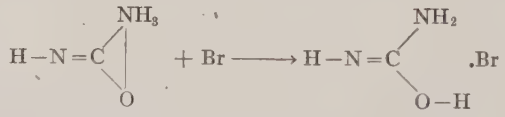
B. By the nitrous acid, directly:

$$H—N=C=O + HNO_2 \longrightarrow N_2 + CO_2 + H_2O.$$

The relative proportions of these different reactions depend upon various factors and for this reason it is not possible to use the method for quantitative purposes.[1] The practical interest in the reaction is, however, that in the quantitative determination of amino-acids by the gasometric method of Van Slyke,[2] the basis of which is the deaminization of these amins by nitrous acid, freeing N_2, which is measured over water, some nitrogen may arise from any urea which is present unless conditions are carefully controlled.

2. The Hypohalogenite Reaction: By hypohalogenites we mean such compounds as hypochlorite and hypobromite. The reactions are as follows:

(*a*) Halogenation:



(*b*) Hydrolysis and oxidation:

The bromin in (*a*) is derived from the solution of bromin-sodium hydroxid used. The O_2 is derived from the readily decomposed NaOBr. The hypobromite method has been used for clinical purposes. The CO_2 is absorbed by a caustic alkali, NaOH or KOH and the nitrogen is read as a column over water. This volume, expressed for standard temperature and pressure, is then converted into mass of nitrogen by the usual procedure discussed under the gasometric method of Van Slyke.[3] This procedure is found in the usual textbooks.[4] The hypohalogenite method is superfluous and far less exact than the urease method. "In spite of its shortcomings . . . this method is probably more largely used than any other for the estimation of urea."[5] Its use, nevertheless, should not be encouraged because:

[1] Conversely, however, it is possible to determine the concentration of HNO_2 by the action of urea on this acid.

[2] Page 294. [3] Page 294. [4] Mathews, cited on page 407.

[5] Werner, page 700.

1. The results are inaccurate, even for clinical work. The results given by the hypobromite method more closely approximate the total nitrogen of the urine, owing to the fact that nitrogen is obtained not alone from urea, but from other nitrogenous constituents of the urine.

2. The urease method demands no more apparatus, no more time for making the determination, and does not demand the gas analysis calculations of the gasometric method.

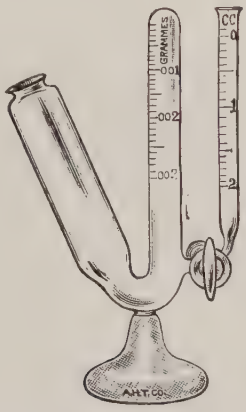


Fig. 212.—Doremus-Hinds' ureameter. Hypochlorite solution is placed in the wide, ungraduated tube; then the tube is tilted until the fluid fills the larger graduated tube. Finally, 1 or 2 mls. of urine are added from the small burette. The nitrogen is read in grams per ml. The error of the method is that total nitrogen rather than urea nitrogen alone, is approximated.

The Clinical Significance of Urea.—Urea is an exogenous product, that is, one which varies in amount with the character of the food.[1] A high protid diet leads to an increase in urea excretion; a low meat diet to a lowered output of urea. This is shown by the following table (Folin):

	High protid diet.	Low protid diet.
Total nitrogen in urine.....	16.8 gs.	3.60 gs.
Urea nitrogen.....	14.7 gs. (87.5 per cent.)	2.20 gs. (61.7 per cent. t. n.)

The average normal urea excretion is about 85 to 90 per cent. of the total nitrogen, 10 gs. urea nitrogen corresponding to about 30 gs. dry urea, per twenty-four hours. During fasting, urea falls, both absolutely and relatively to the total nitrogen. There is a corresponding

[1] The terms "exogenous" and "endogenous" were used by Burian and Schur (Med. Klin., 1905), but the concept was extended by Folin in the same year.

rise in ammonia, due to the hydrolysis of the cyanic acid, according to the following equation:

$$\underset{\text{Cyanic acid.}}{H{-}N = C = O} + \underset{\text{Water.}}{H_2O} + \underset{\text{Acid.}^1}{HA} \longrightarrow \underset{\text{Ammonium salt.}}{NH_4A} + \underset{\text{Carbon dioxid.}}{CO_2}$$

In all acidosis there is this decrease in urea and a corresponding rise in ammonia. Since acidosis occurs on fasting, the above equation represents a modification of the usual course of nitrogen metabolism, the normal manner being the formation of urea from the cyanic acid derived from the protid.[2]

Pathologically, urea shows variations:

Urea excretion is increased:

In fever; diabetes and polyuria in general; following the crisis and consequent absorption of the exudate in pneumonia, in pleurisy with effusion, etc. In exophthalmic goiter and in many cases of cancer urea is increased. In general, heightened metabolism involves increased elimination of urea.

Urea is decreased:

In all cases of urinary retention, like later stages of nephritis; hepatic disorders, as in cirrhosis, acute yellow atrophy, etc.; in psychic states, like paresis and melancholia. Exercise lessens urea excretion.

Urea is variable:

In acute nephritis, chronic nephritis, hysteria, and in excessive exercise.

The urinary urea is derived from the blood, which transports it from the organs where it is made. The liver is largely responsible for this synthesis, but other organs, especially the kidneys, partake of this function, and it is probable that urea formation occurs throughout the body as a typical function of all living cells. Later, a quantitative expression for the relation between urea in the urine and in the blood will be given.[3]

The *origin of urea in the liver* has been demonstrated by recent workers.[4] Investigators at the Mayo Foundation[5] removed the liver

[1] An acid is composed of H^+ and an anion like Cl^-, COO^-, etc. For brevity, the anion is expressed in the above equation as A.

[2] Concerning the manner in which cyanic acid is derived from protid as a step in the formation of urea from meat, see page 707.

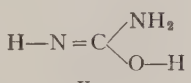
[3] Chapter XVI. See editorial, Jour. Amer. Med. Assoc., vol. 80, p. 1073, 1923.

[4] Morgulis, S. (page 24), Journ. Biol. Chem., vol. 66, page 353, 1925.

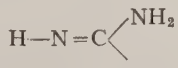
[5] Bollman, J. L., Mann, F. C. and Magath, T. B. (Mayo Foundation, Rochester, Minn.), Amer. Journ. Physiol., vol. 69, page 371, 1924. The origin of urea is fully treated.

in an experimental animal and found that the production of urea was suppressed. Later Morgulis, upon quite different testimony proved that the liver is responsible for the formation of this substance. This testimony was taken from the fact that when the urea and the amino-acid content of the blood are determined following a protid meal, the increase in urea *precedes* the increase in amino-acids. Two processes must occur if this be the case: (1) Deaminization of the amino-acids and (2) systhesis of urea. Folin and Berglund previously pointed out that in such a case, the liver must be assigned the task of performing these functions. In furthur experiments, Morgulis studied the urea and other nitrogen concentration in the blood of Eck fistula[1] dogs and found that the urea nitrogen was very low (0.003 to 0.006 g. urea nitrogen contrasted with a normal of 0.011 to 0.034 g. per 100 mls. blood), according to the time of feeding. These observations point to one conclusion: The liver is the seat of urea formation. Morgulis, however, warns that two processes (named above) have to be considered in any such discussion, namely deaminization of amino-acids and urea synthesis, and that the chief rôle of the liver may be the latter.

How Urea is Synthesized in the Body.—Reference to page 259 will reveal a radicle known as guanidin radicle in the formula for the amino-acid arginin, which bears close relations to urea, as shown by the following formulæ:



Urea.

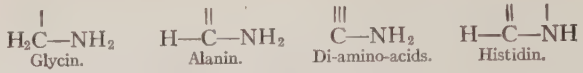


Guanidin nucleus in arginin.

It has been known for a score of years that urea is derived from this amino-acid by the separation of the guanidin nucleus from the remainder of the α-amino-chain. No other amino-acid presents a configuration which permits us to derive urea from it in such a simple manner. Creatin, although not an amino-acid, contains this radicle, creatin being methylated guanidin acetic acid, but there is no reason other than this for believing that urea could be derived from creatin. The same may be said of the anhydrid, creatinin. However, it is necessary to assume that urea is derived from amino-acids in some manner, because increased intake of amino-acids results in increased elimination of urea. In the amino-acids derived from the digested

[1] Eck, G. W., Prussian experimental surgeon, died 1848. The fistula is made by a side-by-side anastomosis of the vena cava inferior and the portal, thus cutting the liver from the circulation.

meat, these configurations are found, leaving off the carboxyl and the aliphatic chain:

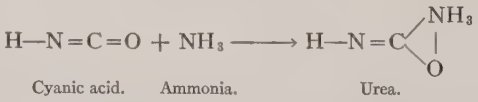


These formulæ represent the various ways in which carbon and nitrogen are associated in the amino-acids. It is easily seen that from such configurations, cyanic acid may arise by oxidation: O=C=N—H (or H—N=C=O, as usually written). Then, by hydrolysis, urea is formed:

$$2HNCO + H_2O \longrightarrow CON_2H_4 + CO_2.$$

The last reaction is readily accomplished in the test-tube at the temperature of the human body, 37° C.

Besides the amino-acids,[1] which are the normal sources of urea, other substances containing ammonia, or the ammonia radicle, may form urea in the body. Ammonium chlorid administered to rabbits is followed by the excretion of practically the same amount of nitrogen as urea as the nitrogen given in the form of NH_4Cl. It seems that ammonia must be liberated in such cases. Carnivores excrete an acid urine. When ammonium chlorid is fed to a dog, urea is not increased, but the ammonium chlorid is excreted as such. Ammonia is not freed from its union with the chlorion. Does a similar process occur in the organism in which ammonia is deaminized from amino-acids? Such an origin is probable, the ammonia then conjugating with cyanic acid to form urea as follows:[2]



It is not known how much deaminization occurs, but it is probable that from the data of metabolism experiments, some of which have been discussed,[3] that the ammonia is removed from the fatty acid in the amino-acids, leaving the acid to be utilized as glucid.

Therapeutic Uses of Urea.—1. As a functional test for renal efficiency: Urea is a normal constituent of the urine and when admin-

[1] See Fiske, C. H., and Sumner, J. B. (Harvard Medical School and Cornell Medical, Ithaca, respectively). The Importance of the Liver in Urea Formation from Amino-acids, Jour. Biol. Chem., vol. 18, p. 285, 1914.

[2] Gottlieb has recently failed to identify cyanic acid in blood (Bioch. Journ., vol. 20, page 1, 1926). He believes that the explanation given above for urea synthesis cannot be valid because of the toxicity of cyanic acid, but many toxic substances appear in intermediate metabolism.

[3] Page 539.

istered, it is recovered from the urine quantitatively in the same amounts, if the kidney functions properly. If, however, the kidney is deranged, as in mercuric chlorid poisoning, or in the case of hypoplasia of one or both kidneys, the urea will not pass through readily, but is held back ("retained") in the blood and tissues.[1] Quantitative determination of the urea content of samples of urine obtained by catheterization of each ureter will disclose the malfunctioning of one kidney, the other being normal, as in the case of contracted kidney, etc. In pregnancy there is at times occlusion of one of the

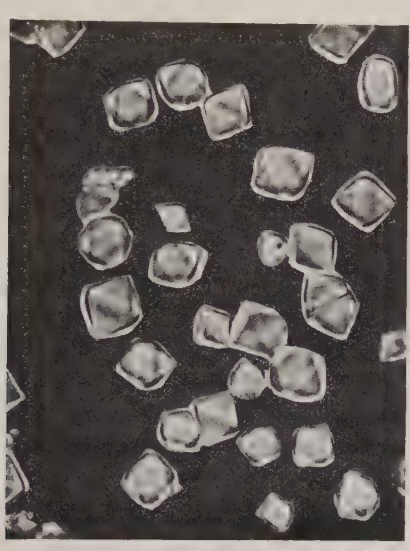


Fig. 213.—Urease crystals. (Kindness of Prof. J. B. Sumner.)

ureters by pressure of the fetus and such a procedure as the one just described frequently suffices to detect the nature of the difficulty without necessitating surgical intervention.

2. As a diuretic: This has been discussed on page 681. From 20 to 40 gs. of urea are taken by mouth, although urea may be administered hypodermically. It is possible to administer urea too liberally, for if the blood urea rises from the normal of 12 mgs. per 100 mls. of whole blood to eight or ten times that figure, lassitude, fatigue, or more serious symptoms may appear. The diuretic effect of urea is due in part to the fact that it is a "no threshold" substance, that is, it behaves indifferently in passage through the kidney membranes and

[1] Coal-tar dyes are, likewise, retained by a diseased kidney and this affords a basis for clinical tests. The dye phenolsulphonephthalein is used (page 65). For cystoscopic work, indigo-carmin (indigo-sodium-disulphonate) is used.

is very slightly absorbed back in the tubules and then only by diffusion.

In a later section, methods for detecting disease of the kidney by means of the tests mentioned above will be given (Chapter XVI).

Quantitative Determination of Urea in the Urine (Method of Folin).—Principle: Urea is converted into ammonium carbonate and by-products by the action of the enzyme urease[1] found abundantly in certain Oriental beans, like the soy-bean[2] and the jack-bean. The ammonium carbonate is then broken up, freeing ammonia, which is quantitatively determined by treating the preparation with Nessler's solution (ammonia in the presence of Nessler's solution,[3] causing the formation of a reddish liquid), and comparing it in a colorimeter with a solution of ammonia (from ammonium sulphate) of known strength, likewise treated with Nessler's solution. The mode of action of urease is as follows:[4]

1. Absorption of the urea to the colloidal urease, thus concentrating the urea.

2. Dissociation of the urea into cyanic acid, $H-N=C=O$ and ammonia, the latter being held by the urease.

3. Hydrolysis of the cyanic acid: $H-N=C=O + 2H_2O = NH_3 + H_2CO_3$. The products, ammonia and carbonic acid, form the acid carbonate, NH_4HCO_3.

4. This acid carbonate unites with NH_3 to form ammonium carbonate,

$$NH_4.H.CO_3 + NH_3 = (NH_4)_2CO_3.$$

The ammonium carbonate, thus formed, simply separates ammonia in the urine from other substances. Then, by the aid of caustic soda contained in the Nessler's reagent, the ammonia is liberated:

$$(NH_4)_2CO_3 + 2NaOH = 2NH_3 + CO_2 + 2H_2O + Na_2CO_3.$$

[1] Miquel, P., Le ferment soluble de l'urée, Comptes rendus (Paris), vol. 3, p. 397, 1890. Sumner (page 24) has been able to crystallize urease.

[2] Takeuchi, T., Urease in Higher Plants, Jour. Coll. Agric., Tokio (Japan), vol. 1, p. 1, 1909.

[3] The chemistry of Nessler's solution is as follows: "Millon's Base" ($Hg_2N.OH.2H_2O$) is formed when mercuric oxid is treated with ammonia. In the presence of iodin the iodid of Millon's Base is formed $(OHg_2)NH_2I$. This compound is the cause of the red color of Nessler's solution in the presence of ammonia. The solution is a mercuro-potassium iodid. Millon's base is constructed somewhat like ammonium hydroxid:

$H_4N.OH.2H_2O$	$Hg_2N.OH.2H_2O$
Ammonium hydroxid.	Millon's base.

[4] Fearon, W. R., Urease, Part II. The Mechanism of the Zymolysis of Urea, Biochem., Jour., vol. 17, p. 800, 1923.

The ammonia thus produced forms the red substance (OHg_2)-NH_2I, referred to above. In order that the enzyme act favorably, a neutral reaction must be maintained, and this is done by adding a buffer composed of a phosphate mixture (page 66).

Reagents (Appendix): Nessler's solution.
Standard ammonium sulphate solution.
Urease preparation.
Buffer mixture.

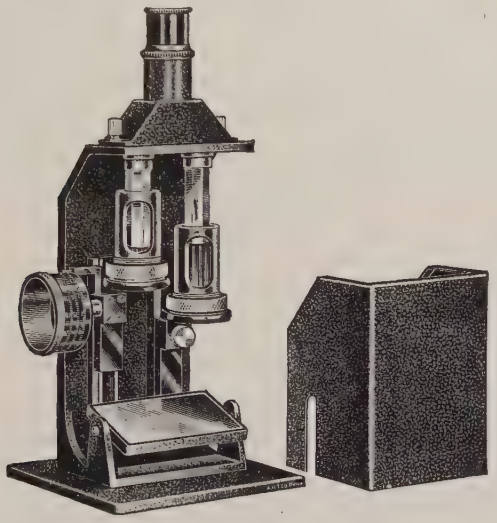


Fig. 214.—Colorimeter for biochemical analyses. The calibrated drum on the reader's left records the excursions of the "unknown" cup, up and down. The "standard" is placed in the opposite cup.

Procedure: 1. Pipette 10 mls. of the urine into a 100-ml. volumetric flask and dilute to the mark with distilled water. This makes a 1:10 solution.

2. Using a 1-ml. Ostwald-Folin pipette, place 1 ml. of the diluted urine in an ordinary test-tube and add 1 drop of the buffer mixture and also 1 ml. of the urease solution.

3. Leave the tube in a thermostat at 50° C. for eight minutes or at room temperature (20° C.) for fifteen minutes or longer.

4. Pour the contents of the tube into a 200 ml.[1] volumetric flask and add about 150 mls. of distilled water.

5. Preparation of the standard: In a similar 200 ml. volumetric flask place 10 mls. of the standard ammonium sulphate solution. Then dilute, as before, with distilled water to about 150 mls.

[1] See note 1, page 698.

6. Pour into each of two small beakers 20 mls. of Nessler's solution. Then simultaneously pour the contents of one beaker into the unknown flask and the contents of the other into the standard flask. Mix, and dilute to the mark in each case. Stopper and invert the flasks several times. Discard about 5 mls. of liquid from each flask to insure uniform samples.

7. Add the standard solution to each of two colorimeter cups until they are about half-full and place each under the colorimeter plungers. Raise the left cup[1] until the reading on the scale is exactly 20 mms. Then match the colors by wheeling up the right-hand cup by means of the calibrated drum. The two readings should agree within 0.2 millimeter.

8. Now pour the standard solution from the right-hand cup and refill the cup rinsing once with liquid from the unknown flask.[2]

Compare.

9. Calculation:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} = \frac{\text{Concentration of unknown}}{\text{Concentration of standard}}.$$

The Reading of standard is 10 mms.

Reading of the unknown is n mms.

Concentration of the unknown is x mgs.

The Concentration of the standard is 1 mg.[3]

Since the urine was diluted 1:10, the result must be multiplied by 10. This gives the milligrams of nitrogen in 1 ml. of the urine as voided. To express the result in terms of per cent. multiply by 100. To express the result in terms of twenty-four-hour quantity, multiply the quantity for 1 ml. by the number of milliliters of urine voided in twenty-four hours.

Method of Marshall.—This method is adapted to rapid clinical work. Principle: The theory is practically the same as that for the

[1] The description is given for the Bausch and Lomb biological colorimeter.

[2] Care must be taken that bubbles do not collect on the lower face of the prism plungers. These may be obviated by inclining the cups as they are placed in position.

[3] By concentration is meant mass per volume. The standard solution is so made that each 10 mls. contains 1 mg. of nitrogen. That is, the ratio between the molecular weight of the sulphate and the molecular weight of the nitrogen contained in $(NH_4)_2SO_4$ is:

$$\frac{132.144}{28.0} = 4.716.$$

For a solution in which 10 mls. contains a milligram of nitrogen, obviously 0.4716 g. of $(NH_4)_2SO_4$ must be used. In making the standard solution used here, 0.4716 g. of pure ammonium sulphate crystals was dissolved and made up to 1000 mls. of solution with water.

foregoing method. The difference is that, instead of determining the ammonia by Nesslerization, the ammonia is titrated with standard acid solution.

Reagents: Urease solution, as in the preceding method.

Toluene, $C_6H_5.CH_3$, for preserving the solutions against bacterial action.

Decinormal acid solution, page 95.

Methyl orange indicator, Appendix.

Procedure: Pipette 5 mls. of urine into each of two "carbon dioxid flasks of 200- to 300-mls. capacity (Fig. 215). Label one flask "Experiment" and the other "Control." Dilute the contents of each flask with 100 mls. of distilled water. Add to each 2 mls. of toluene. To flask "Experiment," add 2 mls. of urease solution.

Fig. 215.—"Carbon dioxid" flask, recommended as a substitute for the ordinary beaker in biochemical work when a volatile substance is to be stored for a time; the flask may be stoppered.

Stopper both flasks. Let the preparation stand for the following periods according to temperature.

At room Temperature, 20° C., minimum six hours or overnight.
Temperature, 40° C., sixty minutes.
Temperature, 50° C., fifteen minutes.

At the end of the period add 2 mls. of the urease solution used for the experiment to the "Control" flasks, and then titrate each flask separately with the standard acid, with methyl orange as indicator.

Calculation: Subtract the reading of the "Control" from that of the "Experiment" and multiply by the weight of nitrogen in 1 ml. of the 0.1 normal solution.[1] The result gives the number of grams of urea nitrogen in 5 mls. of the solution. To express in per cent., multiply by the number of 5's in 100, that is, 20. To express in terms of twenty-four-hour quantity, divide by 5 to obtain the value for 1 ml. and multiply by the twenty-four-hour quantity. By multiplying the burette reading obtained by titrating with 0.1 normal HCl

[1] For the derivation of this factor see page 290. The factor for 0.1 normal solution is 0.0014 g.

by 0.6, you obtain the grams of urea as dry urea (not nitrogen) in a liter of urine, since 1 ml. of 0.1 normal HCl corresponds to 3 mgs. of urea.

Ammonia.—The urine contains about 0.5 g. of ammonia nitrogen, corresponding to 0.7 g. as NH_3 in an average sample taken over a period of twenty-four hours. In a three-hour morning period, about 0.065 g. urea nitrogen is excreted (Folin). The ammonia is free ammonia; that is, it is readily volatilized by an air current and readily absorbed, chemically.[1]

Test for ammonia: The Nessler reaction is a faithful test for the presence of ammonia (page 710).

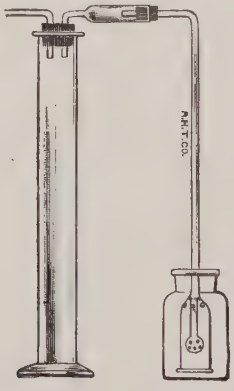


Fig. 216.—Folin's ammonia apparatus for larger quantities of urine. A known quantity of urine is placed in the cylinder and the ammonia is blown into the known amount of acid of known normality in the bottle. The special tube in the bottle is designed to afford rapid means of absorption.

The *clinical significance of ammonia* applies largely to the acid-base regulation in the body. Benedict finds that the ammonia, obtained from urea in the kidney, acts as an agent to save the loss of basic radicles from the alkali reserve of the blood and tissues, by replacing them before the acid radicles are excreted.[2] Thus, BH_2PO_4, consisting of Na, K, etc., united to the radicle PO_4, may carry away a relatively large amount of basic radicles, like Na, K, etc., but in the kidney, before they are lost, ammonia may replace them. Thus, ammonia conserves the alkali reserve. We have mentioned the inverse proportion of urea and ammonia in the urine; the rise of ammonia is accompanied by the lowering of the urea. It is for

[1] It probably exists as NH_4OH, or as a loosely bound compound in equilibrium with it: $NH_3 + H_2O \leftrightarrows NH_4OH$; $2(NH_4OH) + H_2CO_3 \leftrightarrows (NH_4)_2CO_3 + 2H_2O$, etc.

[2] Page 588.

reasons such as these that in acidosis ammonia increases. In fact, acidosis probably occurs when the kidney cannot make ammonia rapidly enough to replace the basic radicles owing to the overproduction of acid in the body, and thus there is a loss of alkali reserve.

Pathological variations in urinary ammonia occur in various forms of acidosis, like that which occurs in diabetes mellitus, in diminished oxidative powers of the body other than in diabetes, as in acid intoxications, in dyspnea, in the pernicious vomiting of pregnancy, in diseases of the liver such as acute yellow atrophy, cirrhosis, and malignancy. Such conditions involve an increased output of ammonia. On the other hand, there may be a decreased elimination of ammonia in nephritis, in hypochlorhydria, and in achylia gastrica.[1] It is customary to administer sodium bicarbonate in cases of increased ammonia excretion and this causes a lowering of the ammonia output as long as the effect lasts. The use of $NaHCO_3$, however, in such cases is limited on account of attending difficulties like sodium intoxication. In fasting there is an increase in ammonia in the urine. Drinking large quantities of water increases the elimination of ammonia. A large diet of fat reduces its excretion in the urine, probably because of the chemical binding of ammonia to the fatty acid radicles. The most important clinical use of urinary ammonia determinations is in diabetes mellitus and in the toxemias of pregnancy.

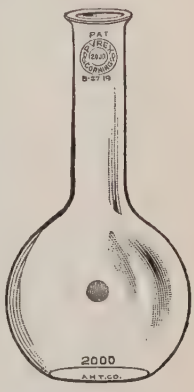


Fig. 217.—Florence type flask.

Quantitative Determination of Ammonia in the Urine (Method of Folin).—Principle: Powdered artificial silicates, resembling in chemical structure the naturally occurring zeolites,[2] share with them the power of having the loosely combined sodium of their molecule replaced by a metal or radicle like Mg, Ca or NH_4. The equation in the case of ammonia is:

$$\underset{\text{Sodium "permutit."}}{Na_2(Al_2Si_2O_8)} + \underset{\text{Ammonias.}}{2NH_4OH} = \underset{\text{Ammonium "permutit."}}{(NH_4)_2(Al_2Si_2O_8)} + \underset{\text{Sodium hydroxid.}}{2NaOH}$$

[1] Page 449.

[2] The zeolites are hydrated sodium-aluminum-calcium silicates found in various soils, especially those which have been weathered. The artificial zeolites, sold under the trade name "Permutit" (pronounced by the Permutit Company "per-mu'-teet"), are used in softening water for industrial purposes. Other artificial zeolites are known as borromite. For a discussion of the chemistry of permutit see Frankforter, C. J., and Jensen, F. W. (University of Nebraska, Lincoln), Industrial and Engineering Chemistry, vol. 16, p. 631, 1924.

In Folin's method, ammonia replaces sodium. The reaction is a chemical replacement and not a physico-chemical "adsorption." The ammonia is attached to the powder, which is washed free from urine and substances, like urinary creatinin which interfere with the reaction.

Procedure: Pour about 2 gs. of the specially prepared permutit for ammonia determinations[1] into a 100-ml. volumetric flask. Add not more than 5 mls. of water and 1 ml. of undiluted urine accurately measured by means of the Ostwald-Folin pipette. After making sure that none of the urine has remained in the neck of the flask, agitate the contents for not less than five minutes.[2] Add about 50 mls. of distilled water, rinse it about to catch all of the powder, and then let it settle with the flask inclined considerably to one side. Decant the liquid down to the heavier powder, neglecting the fine suspension. Repeat once or more in case there is bile[3] in the urine. Now finally dilute to about 25 mls. with distilled water, add 1 ml. of 10 per cent. NaOH solution, mix, and then dilute to about 75 mls. with distilled water. While rotating the contents of the flask, introduce 10 mls. of Nessler's solution (Appendix). Then fill to the mark, mix, and compare with a standard prepared by transferring 5 mls. (equivalent to 0.5 mg. nitrogen) of the standard $(NH_4)_2SO_4$ solution to a second 100-ml. volumetric flask and add 1 ml. 10 per cent. NaOH. Dilute to about 75 mls. and mix. The standard must be "nesslerized" simultaneously with the unknown.

Calculation: This is similar to that for urea.

Method of Sumner.[4]—In place of permutit, cupric hydroxid is used. It precipitates creatinin of the urine, which interferes with the determination of the ammonia. The ammonia may then be determined without separating it.

Reagents (Appendix): (1) Cupric sulphate solution.
(2) Sodium hydroxid solution.

[1] Obtainable from the makers, The Permutit Co., 440 Fourth Avenue, New York, N. Y., or from the Philadelphia Office, Liberty Building, Philadelphia, Penna. When ordering, "Folin's special" should be specified; this is a more homogeneous powder, which settles readily.

[2] A simple device for shaking, available everywhere, is to tie the flask to a water-tap and slightly open the faucet; the stream causes a gentle movement of the contents of the flask.

[3] The presence of bile-salts causes a shift in the electrolytes, thus interfering with the fixation of the ammonium radicle. Compare page 116 concerning the effect of bile-salts on surface tension.

[4] Page 24. See Jour. Biol. Chem., vol. 34, p. 37, 1918.

The reaction concerned is the production of cupric hydroxid:

$$Cu\overline{|SO_4 + 2\,Na|}OH = Cu(OH)_2 + Na_2SO_4.$$

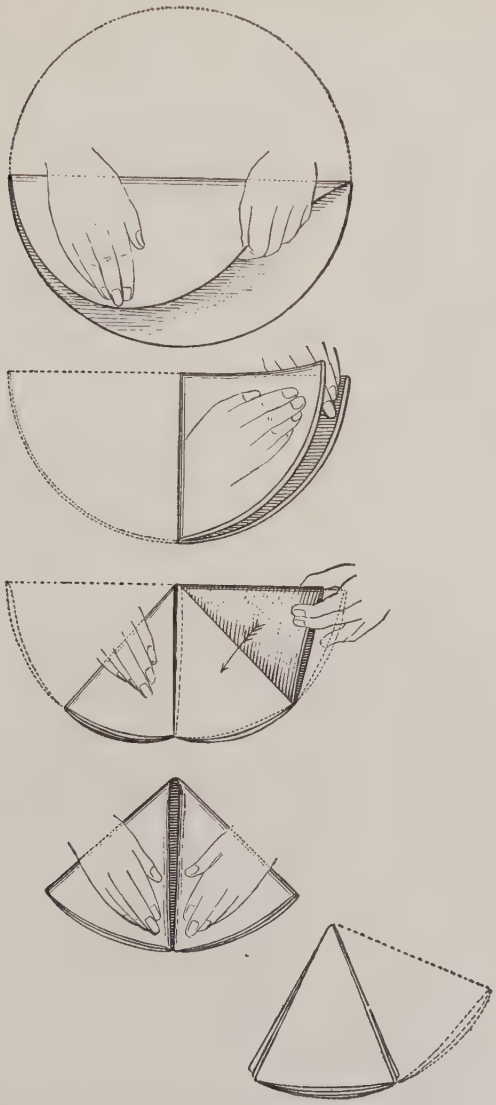


Fig. 218.—An important biochemical procedure—folding a filter to be used with substances that filter with difficulty. The creases cause the paper to stand out from the glass walls of the funnel.

Procedure: Pipette into a large test-tube (200 x 20 mms.) exactly 10 mls. of the special cupric sulphate solution (1), 15 mls. of urine, and 10 mls. of the special NaOH solution (2). Close the tube

immediately with a rubber stopper and shake vigorously, in order to insure complete precipitation of the copper. Filter through a dry filter (covering the funnel with an evaporating dish to prevent concentration). Transfer 5 ml. of the filtrate to a 100-ml. volumetric flask and add 1 drop of a saturated solution of Rochelle salt to dissolve any copper which may have become precipitated. Dilute with 20 mls. of distilled water and "nesslerize" with 10 mls. of the Nessler solution used in the Folin total nitrogen procedure on page 698. Compare with a standard ammonium sulphate solution, 10 mls. of which contain 1 mg. of nitrogen.

Calculation: The calculation is exactly similar to those of the foregoing methods, except that it is necessary to multiply by 7, since only 5 mls. of the filtrate were taken, to obtain amount in 15 mls. of urine.

Method of Ronchèse, Modified by Malfatti.[1]—Principle: This is an adaptation of the method referred to[2] as formol-titration in the quantitative determination of amino-acids. The chemical basis is the same for ammonia, namely, the liberation of acid from a neutral solution of ammonium salt and the titration of the acid by standard alkali:

$$4NH_4Cl + 6H.CHO = N_4(CH_2)_6 + 4HCl.$$

Reagents: Neutralized formalin solution (page 292).

Decinormal or 0.05 normal alkali, as required.

Procedure: Pipette 10 mls. of urine into a small evaporating dish and add 3 drops of phenolphthalein solution (indicator). Titrate with standard alkali to neutrality.[3] Now add an excess (10 mls.) of the neutral formalin solution, mix, and titrate again to neutrality with the standard alkali. Assuming the alkali to be decinormal, the calculation is made by multiplying the titration figure by 0.0014, which gives the number of grams of ammonia nitrogen in the amount of urine taken (10 mls.). Percentage ammonia is given by multiplying by 10. The twenty-four-hour quantity is obtained by dividing by 10 and multiplying by the total twenty-four-hour quantity.

The error in the method is the incorporation of the amino-acid nitrogen along with that from ammonia. The normal urine contains a small amount of amino-acids, but this amount is increased in certain diseases, like acute yellow atrophy of the liver, and in such

[1] Ronchèse, Bull. Soc. Chim. de France, volume for 1907, I, p. 900. Malfatti, Zeitschr. f. anal. Chem., vol. 47, p. 273, 1908.

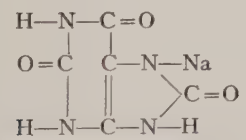
[2] Page 291.

[3] This figure may be rejected unless it is desirable to use it as a rough indication of the acidity of the urine. See, however, page 687.

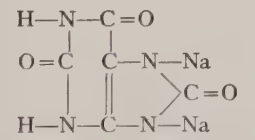
cases the ammonia figure derived by the method just described would be considerably increased over its actual value. The amino-acid nitrogen is readily determined by the Van Slyke gasometric method,[1] or the Folin procedure,[2] and must be subtracted from the ammonia quantity obtained by the Ronchèse-Malfatti method just described, if accurate results are desired.

Uric Acid and Urinary Purins.[3]—Uric acid is excreted in normal human urine in fairly constant amounts, of about 0.2 g. as uric acid nitrogen corresponding to 0.60 g. of uric acid (dry).[4] In addition to this substance, other purins occur in the urine, amounting to about 0.05 g. as crystalline substance in twenty-four hours. These purins are methyl-purins (caffein, theobromin, theophyllin, etc.) derived from beverages like tea, coffee and chocolate. On a diet in which purins are excluded, a so-called "purin-free diet" only these purins appear. On a purin-rich diet, small quantities of amino-purins and hydroxy-purins appear in the urine. The purin bases of the normal urine are exogenous in origin. Uric acid is both exogenous and endogenous, and represents the end-product of the metabolism of the purin bases in the nuclei of the cells and probably some uric acid derived from the purins of the food. The constancy with which the kidney excretes uric acid day after day on varying diets is indicative of its derivation chiefly from the tissues, rather than from the foods.[5]

The chemistry of the purins has been discussed.[6] Amino-purins, guanin and adenin, occur in the nucleoprotids of the cell nuclei and are oxidized in the liver to the oxypurins, hypoxanthin, xanthin, and uric acid. Urates are defined as salts of uric acid. Sodium urate is found in the blood and urine in greater quantity than other salts of uric acid. The formula for sodium urate is as follows (two forms):



Mono-sodium urate (acid urate).



Di-sodium urate (neutral urate).

[1] Page 294. [2] See later in the present Chapter.

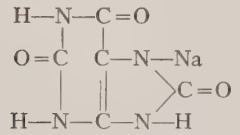
[3] Benedict, S. R., Uric Acid in Its Relations to Metabolism, Harvey Lecture, Jour. Lab. and Clin. Med., vol. 2, p. 1, 1916.

[4] Hanzlik, P. J. (Leland Stanford, Jr., University School of Medicine, San Francisco, California), and Hawk, P. B. (see page 452 note 1). Jour. Biol. Chem., vol. 5, p. 355, 1908, give 0.59 g. uric acid as the average normal excretion per twenty-four hours.

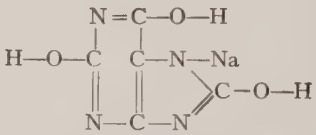
[5] A brief statement of the modern aspect of purin metabolism is given in an editorial in the Jour. Amer. Med. Assoc., vol. 82, p. 1865, 1924.

[6] Pages 332 and 370.

In the urine the most common urate is *mono-sodium urate*, but owing to the low degree of acidity endowed this compound by the hydrogen attached to the atoms 1 and 9,[1] the substance reacts practically neutrally. Free uric acid receives its acidity from the hydrogens on atoms number 3, 9, 1, and 7,[2] and the low degree of acidity donated by the hydrogens on atoms 1 and 7 leaves the ordinary urinary uric acid with a reaction like that of a very weak di-basic acid that is practically neutral in aqueous solution. The urates seem to be differently formed under certain circumstances. In gout, the mono-sodium urate is usually the unstable "α-urate" of Emil Fischer (contrasted with the β-urate):



α-urate, lactam, unstable urate of gout.



β-sodium urate, lactim, stable, of normal urine and blood.[3]

Urates are precipitated as non-crystalline mon- and di-ammonium urate by saturating the urine with ammonium chlorid.

Uric acid, $C_5H_4N_4O_3$, or 2, 6, 8, tri-oxy-purin, in pure crystalline condition, appears as minute rhombic prisms. When impure, the normal crystalline shape is distorted into various "whetstone," "dumb-bell," and rosette shapes. In such form uric acid crystals generally appear in the microscopic examination of the urine. The solubility of uric acid in pure water at room temperature (20° C.) is low, 1 : 40,000 parts of water. In boiling water, the solubility is increased, 1:1600. With the exception of ammonium acetate, borate, and carbonate, alkalies dissolve uric acid. With concentrated sulphuric acid, uric acid forms a sulphate which is readily hydrolyzed by introducing water. Precipitants of uric acid are the so-called alkaloidal reagents, picric acid, mercuric chlorid, silver nitrate (ammoniacal), phosphotungstic acid, and lead acetate. Both uric acid and the urates exert reducing powers on cupric and other solutions; Fehling's solution is reduced, but Benedict's qualitative reagent is affected only on contact with uric acid for some time.

[1] For the nomenclature of purin rings, see page 332. Concerning the matter of acidity of uric acid, see Cohen, J. B., Organic Chemistry for Advanced Students, Part III, Synthesis, London, Edward Arnold & Co., 4th ed., 1923, p. 120.

[2] Arranged according to their effectiveness.

[3] Solubility of β-sodium urate in blood serum, 8 mgs. per 100 mls.; of α-urate, 18 mgs. per 100 mls.

Tests for Uric Acid: 1. *Phosphotungstic Acid Test of Folin.*[1]—Add to the solution suspected of containing uric acid a small pinch of desiccated sodium carbonate, mix, and add 1 ml. of the reagent; a blue color indicates a positive test for uric acid.

2. *Murexid Test.*—Directions for examining a solution: Place 5 mls. of the solution in a porcelain evaporating dish and evaporate to dryness over a free flame and then on a water-bath. Then add to the residue 3 drops of concentrated nitric acid in order to oxidize the uric acid to alloxanin, which is condensed from two molecules of alloxan. Heat the mixture on the water-bath until practically dry and longer if necessary until all the nitric acid has been evaporated. The residue is reddish. While the dish is being heated, prepare a dilute solution of ammonium hydroxid by filling a test-tube with distilled water and adding 3 drops of desk reagent concentrated ammonium hydroxid. Then add several drops of this diluted reagent to the residue in the evaporating dish. In a positive test the color becomes violet red. Next add a few drops of 10 per cent. NaOH solution; the color loses the reddish tinge and becomes bluish violet. The reactions are given on page 373.

Difficulties in obtaining the test are due to:

(*a*) Charring over a free flame; the water-bath must be used, at least to complete the process of desiccation.

(*b*) The drying does not completely remove the nitric acid. It is necessary that all HNO_3 be removed.

(*c*) Using disproportionate amounts of reagents.

The detection of uric acid and urates in urine (Folin): Pipette 2 mls. of urine into a porcelain evaporating dish. Add 1 drop of a saturated solution (10 per cent.) of oxalic acid and evaporate the fluid to dryness on a water-bath. Permit the preparation to cool. Extract the polyphenols, which give the same color as uric acid, by adding 10 mls. of 95 per cent. ethanol. Let stand five minutes and then decant the alcohol by gently pouring it from the residue. Dilute the residue with 10 mls. distilled water and 2 drops saturated solution of sodium carbonate. Stir to dissolve the uric acid in the alkaline medium, add one ml. of the uric acid reagent employed above[1] and wash the fluid from the dish into a small beaker with 20 mls. saturated sodium carbonate solution. A blue color is positive.

Uric acid is prepared from urine by adding to 100 mls. of urine 2 mls. of concentrated HCl, and letting the solution stand overnight or longer; brownish crystals of uric acid appear attached to the sides

[1] Page 909, Folin-Denis Reagent.

and bottom of the vessel. The pigment is a derivative of bile pigments and is known as uro-erythrin.[1]

Clinical interest in the uric acid of the urine has always been marked and research has led to much better understanding of its significance. The investigations of Benedict, Folin, Hunter, and their collaborators in America and of several European workers[2] are particularly noteworthy.

The variations in output of uric acid are:

Physiological.—Diet: The following table, after Lusk from Rockwood,[3] gives a series of observations concerning uric acid excretion over long periods of time:

	G. uric acid, crystal, per twenty-four hours, average.
January	0.308
February	0.305
March	0.315
April	
May	0.321
June	
July	0.313
August	
September	
October	
November	0.298

An extensive series of observations were made by Hanzlik and Hawk[4] on the excretion of uric acid over long periods of time and with controlled diets. The average daily excretion for 10 adults was found to be:

Subject.	Weight.	Average twenty-four-hour excretion of uric acid as such:	As uric acid nitrogen.
F	53.1 kilos.	0.475 g.	0.159 g.
J	76.0	0.520	0.174
B	65.5	0.588	0.196
C	72.2	0.597	0.199
H	66.1	0.608	0.203
A	74.1	0.615	0.206
I	63.3	0.619	0.207
G	68.7	0.644	0.215
K	72.3	0.651	0.218
E	76.7	0.660	0.221

[1] Greek *ouron*, urine, and *erythros*, red.

[2] Among whom may be mentioned Wiechowski, Schittenhelm, Burian, on the Continent, and Onslow in England. For an account of the chemistry of uric acid see Folin, O., and Derick, C. (Harvard Medical School and Peter Bent Brigham Hospital, Boston), Jour. Biol. Chem., vol. 60, p. 362, 1924.

[3] Rockwood, E. W. (Professor of Chemistry, University of Iowa, Iowa City, Ia.), Amer. Jour. Physiol., vol. 12, p. 38, 1904.

[4] See page 719.

The lowest excretion is 0.475 g. uric acid and the highest, 0.66 g. There is no correlation between body weight and uric acid excretion, subject J having considerable weight, but the second lowest amount of excretion.

Folin has shown that the excretion of uric acid varies with the character of the food, although there is no constant relation:

Total nitrogen ingested, 119 gs. Uric acid excreted, 0.37 g. as nitrogen.
Total nitrogen ingested, 0 g. Uric acid excreted, 0.09 g. as nitrogen.

The increase in gland substances (thymus, pancreas, brain, etc.) in the diet causes an increase in uric acid excretion. A high protid diet causes an increased elimination of uric acid, not by producing more uric acid, but by reducing its destruction in the blood and by increasing its passage through the kidney. The same "wash-out" effect of high water or other liquid intake observed in the case of nitrogenous substances in general is also found in the case of uric acid.

Exercise causes a small increase in uric acid excretion.

Pathological Variations.—Uric acid excretion is much increased in fever or in any wasting disease which causes much tissue destruction, as in poliomyelitis; in leukocytosis of whatever kind, and especially in leukemias, due to the destruction of white blood-corpuscles; in pneumonia and empyema, during the absorptive stage following the crisis. Gout demands especial attention.[1] The uric acid of the blood is abnormally high,[2] due to the lowered sensitiveness of the kidney to increase in circulating uric acid; the response of the kidney to changes in uric acid in the blood varies with the individual, but it is pathological in the gouty. Hence, the picture of gout is that of a kidney irresponsive to the demand to increase the elimination of uric acid through the urine. The high blood uric acid is a stimulus to increased destruction of uric acid. This is probably the reason why the kidney passes less uric acid through its tissue into the urine. The accumulation of urates in the cartilages is probably due to the fact that such tissues are, in some manner, abnormal and resemble dead tissue which permits the diffusion of

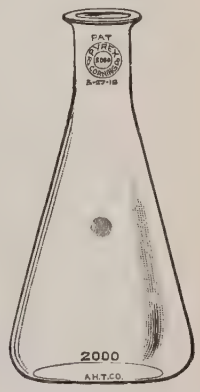


Fig. 219.—Erlenmeyer type of flask.

[1] The most accurate knowledge we possess concerning the chemistry of gout is embodied in the paper of Folin, Berglund, and Derick, mentioned on page 722.

[2] Chapter XVI.

urates into themselves. In rheumatism there is greater excretion of uric acid during the attack, where the fever is high, than during the postfebrile periods.

Uric acid is decreased in anemia (excepting pernicious anemia), lead-poisoning, chronic interstitial nephritis, and sometimes diabetes mellitus (diabetes alternans).

Uric acid calculi occur throughout the urinary tract in many individuals. The calculi are known as "sand," "gravel," and "stones," according to their size and appearance. The type of calculus depends largely upon the reaction of the urine. There is usually a nucleus, or core, around which the calculus is deposited. The core consists of calcium oxalate,[1] or of an organic base, like blood fibrin, epithelial cells, and "casts." The uric acid calculus consists of pure uric acid mechanically mixed with urates and pigments. The various zones may be recognized by fragmenting a calculus and noting the rings of colors, concentric with the nucleus. Calculi may be identified by the tests given above for uric acid and the urates, the calculus being dissolved or suspended in water.

Scheme for Analysis of Urinary Calculi.[2]—Heat a small portion of the powdered calculus on a platinum foil.

I. Burns completely—shows organic matter.

A. With flame: Indicates either Cystin, Urostealith, or Fibrin.

1. **Cystin.**—Dissolve some of the powdered calculus in dilute HCl and divide into two equal portions. To one add 5 mls. H_2O and 5 mls. saturated Na_2CO_3, to second add 5 mls. saturated Na_2SO_3 and 5 mls. saturated Na_2CO_3. Then add 1 ml. phosphotungstic acid to both. A greater depth of color in the second tube than in the first shows cystin.
2. **Urostealith.**—Fuses when heated. Burns with a yellowish flame and with the odor of shellac or benzoin. Soluble in ether.
3. **Fibrin.**—Burns with a yellowish flame and gives off odor of burning hair or horn. Dissolves in KOH and is precipitated by acid. Not soluble in ether.

B. Without flame: Indicates Uric acid, Ammonia Urates or Xanthin.

[1] Other concretions occur in the urine, calcium oxalate being a common form, next in frequency to the uric acid deposits.

[2] Devised by Professor J. M. Looney.

1. First tube under A-1 gives blue color; indicates **urates**.
   a. Portion of powdered material dissolved in KOH and 2 mls. of Nessler's solution added:
      Red color shows **ammonium urate.**
      No color shows uric acid.
2. Powder dissolves in conc. HNO_3 without effervescence. The yellow residue turns orange with alkali, and red on heating gently. Indicates **Xanthin.**

II. Does not burn.

A. Residue dissolves in dilute HCl with effervescence. Indicates carbonate or oxalate.
   1. Original powder dissolves in HCl with effervescence. Shows **carbonate**.
   2. Original powder dissolves in HCl without effervescence. Neutralize residue and add $(NH_4)C_2O_4$: A white precipitate indicates **calcium oxalate.**

B. Powder fuses to hard white mass which dissolves in HCl without effervescence.
   1. Portion of solution treated with 2 mls. of ammonium molybdate solution and then 2 mls. of hydroquinone-sulfite solution (Benedict) added. Blue color shows **phosphate.**
   2. Rest of solution neutralized with NH_4OH leaving it slightly acid; then $(NH_4)_2C_2O_4$ added. White ppt. shows **calcium phosphate.**
   3. Filter. Add excess of ammonium hydroxid. White precipitate shows triple phosphate. **Ammonium magnesium phosphate.**

Quantitative Determination of Uric Acid (Method of Folin, Modified by Benedict).—Principle: Uric acid in a measured amount of urine is treated with a cyanid in order to intensify the color produced by a modified phosphotungstic uric acid reagent; the chief modification of this method is the introduction of arsenic pentoxid to form, and arsenophosphotungstic acid[1] which increases, the specificity of the reagent for uric acid. The colored solution thus obtained is compared

[1] The formula for a compound synthesized by Fremery (M. Fremery, Ber. d. d. chem. Gesellsch., vol. 17, p. 29, 1884) from potassium tungstate and arsenic pentoxid, As_2O_5, is:

$$6WO_3.As_2O_5.4K_2O.$$

The arsenophosphotungstic reagent of Benedict has sodium replacing potassium and, in addition, has phosphoric acid, making, therefore, a substance having a formula similar to the above, but with H_3PO_4 added to it, and with the atoms Na in place of K.

in a colorimeter with a standard solution of uric acid, similarly treated.

Reagents: For the preparation of the reagents see the Appendix.

Procedure: Dilute the urine 1:20[1] by pipetting 5 mls. of the urine into a 100-ml. volumetric flask and adding water to the mark. Mix and pipette 10 mls. of the diluted urine into a 50-ml. volumetric flask. Add 5 mls. of 5 per cent. sodium or potassium cyanid solution

from a burette[2] and 1 ml. of the arsenophosphotungstic acid solution. Mix. After five minutes dilute to the mark, mix by pouring from the flask into a beaker and back again. Rinse out the beaker; finally, compare with a standard made during the five-minute interval as follows: Ten mls. of the standard uric acid solution which contain 0.20 mg. of uric acid are pipetted into a 50-ml. volumetric flask. Add 5 mls. of the cyanid mixture from a burette and 1 ml. of the uric acid reagent. Leave for the same period (five minutes), dilute to the mark, stopper, and mix.

Calculation:

$$\frac{\text{Reading of the standard}}{\text{Reading of the unknown}} \times 2 \text{ gives the mgs.}$$

of uric acid as such in 5 mls. of urine. For per cent., multiply by 20. For twenty-four-hour quantity, take 0.2 of this result and multiply by the figure denoting the twenty-four-hour quantity of urine. Note that the result is uric acid and not uric acid nitrogen.

Method of Folin.—Principle: This method requires a centrifuge to cause the precipitate of uric acid as silver urate to settle. The precipitated silver urate is then freed from interfering polyphenols in the supernatant liquid. Next the silver precipitate is dissolved in the cyanid solution, sodium carbonate is added to permit the development of the color, and the preparation is read in the colorimeter against a standard uric acid solution.

Reagents: See Appendix.

Procedure: Secure two 15-ml. centrifuge tubes and place them in your test-tube rack. Pipette with great accuracy 2 mls. of urine into

[1] Or to such a volume that the uric acid content is about 0.15 to 0.30 mg. per 10 mls. of urine.

[2] Caution!! Two-tenths of a gram of KCN are toxic. Less than 1 ml. of a 5 per cent. solution may be fatal!

each tube. Add about 3 mls. of distilled water and the same amount of special silver lactate solution. Set each tube on a pan of the balance near the centrifuge machine and adjust the balance so that when the tubes are placed in the machine they are in equilibrium. Centrifuge two minutes at high speed (Fig. 142). Then pour from each tube all of the supernatant liquid possible. Prepare the standard as follows: Pipette, accurately, 5 mls. standard uric acid solution into a 100-ml. volumetric flask and add from a burette[1] 1 ml. of the 15 per cent. cyanid solution and 20 mls. of saturated sodium carbonate. Next, to each of the centrifuge tubes add 1 ml.

Fig. 220.—A 15-ml. centrifuge tube, graduated. For mounting when in use, see Fig. 221.

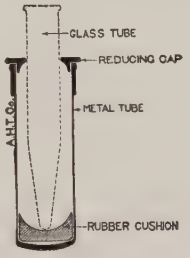


Fig. 221.—Fifteen-ml. centrifuge tube in its situation in the centrifuge cup. The cup is the same as that used with the 50-ml. tube, but a small metal cap (reducing cap) holds the 15-ml. tube in place.

of cyanid solution, dissolve the precipitated silver urate by means of a fine ball-tipped stirring-rod, and fill each tube with distilled water (5 mls.). Pour the contents of each tube into one of two 100-ml. volumetric flasks and add to each flask 20 mls. of the sodium carbonate solution. Grasp the neck of each flask and cause the contents to rotate. While the liquid is in motion add to each flask 5 mls. of the uric acid reagent, and after five minutes dilute the contents of each flask with distilled water to the mark. Stopper, invert, and agitate the contents thoroughly; then immediately pour from each flask into small beakers about 40 mls. of each of the liquids. A heavy sediment appears and a clear, supernatant liquid overlies it in each of the beakers. Pour some of this clear solution from the beaker containing the standard into both the cups and adjust the colorimeter and then replace solution in right cup with that from one of the other beakers. Make comparison. Repeat with the second beaker, which acts as control.

[1] See page 726.

Calculation: 5 mls. of the standard uric acid solution contain half a milligram of uric acid.

$$\frac{\text{Reading of the standard}}{\text{Reading of the unknown}} = \frac{\text{Concentration of unknown}}{\text{Concentration of standard}}.$$

If the standard is placed at 20 mms., then $\frac{20}{n} = \frac{x}{0.5}$. Then $nx = 10$ and $x = \frac{10}{n}$. With the standard at 20, the number of milligrams of uric acid (dry) in 2 mls. of the urine being analyzed is 10 divided by the colorimetric reading of the unknown. Per cent. uric acid is 50 times that figure. The twenty-four-hour excretion of uric acid is obtained by dividing the result for 2 mls. by 2 and multiplying by the figure representing the volume of urine excreted in twenty-four hours. *Discard the solutions containing cyanide by pouring them into the special receiver.*

Method of Morris.[1]—Principle: This method is based upon the procedures of Folin and of Benedict, but precipitation of the uric acid is made with zinc (chlorid), thus avoiding the deterioration of the silver lactate reagent used in the former methods, in daylight; also, "arseno-18-tungstic acid" replaces the arsenophosphotungstic acid reagent of the foregoing methods.

Reagents: See the Appendix for composition of the arseno-18-tungstic acid solution.

Zinc chlorid, 2.5 per cent. aqueous solution.

Procedure: Pipette 1 ml. of urine into a 50-ml. centrifuge tube and dilute with distilled water to about 40 mls. Add 1 ml. of the zinc chlorid solution and mix with a small stirring rod. Add 1 ml. of 10 per cent. sodium carbonate solution and stir. Centrifuge for two minutes, decant the supernatant fluid and dissolve the residue in about 4 drops of 10 per cent. hydrochloric acid solution, using a stirring-rod. Add 5 mls. of distilled water and 10 mls. of 10 per cent. sodium cyanid from a burette. Pour the liquid into a 100-ml. volumetric flask and wash the tube with a small amount of water until the washings, when added to the liquid in the volumetric flask, make a total volume of 60 mls. Prepare the standard by pipetting 1 ml. of the special phosphate standard uric acid solution into a 50-ml. volumetric flask and dilute to about 30 mls. Add 5 mls. of the 10 per cent. sodium cyanid solution from a burette. Placing the 100-ml. and the 50-ml. volumetric flasks side by side before you, add to

[1] Morris, J. L. (died, 1926), Western Reserve School of Medicine, Cleveland, Ohio), Colorimetric Determination of Uric Acid, etc., Jour. Biol. Chem., vol. 50, p. 55, 1922.

each, at the same time, the following amounts of the arseno-18-tungstate reagent: 1 ml. to the 50-ml. flask containing the standard uric acid solution (0.5 mg. uric acid) and 2 mls. to the 100-ml. flask, (unknown). Agitate the contents of the flasks and dilute to the marks. Read in a colorimeter against the standard.

Calculation: There is 0.5 mg. uric acid in 50 mls. of the standard. This corresponds to 1 ml. in 100 mls. of solution. Applying this to the general formula given above, the concentration of uric acid in percentage of twenty-four-hour quantity of urine may readily be made.

Creatin and Creatinin.—The chemistry of these compounds has been discussed.[1] Creatin is methyl-guanidin-acetic acid and creatinin is its anhyrid. Creatin is present in the urine only at certain stages of life, while creatinin is a normal and the most characteristic component of the urine. Creatin at least is both endogenous and exogenous in origin. However, the knowledge of the origin, function, and meaning of these components is less certain than that concerning any other substance in the urine. One reason for this is that prior to 1904 no method for accurately and rapidly determining creatinin had been developed, and it is only by virtue of the work of Folin published in that year that progress has been made. The method for creatin followed. The close chemical relation between creatin of the muscle and of the blood and creatinin of the urine suggests that one is derived from the other; yet up to the present time, no unchallenged proof has been offered for this theory. Myers[2] and collaborators have shown the constancy in amount of muscle creatin and ascribed to this factor the constancy in elimination of urinary creatinin. The creatinin content of muscle is greater than that of any other tissue. When creatin is added to muscle undergoing autolysis,[3] the creatin is converted to creatinin at a constant rate, which is about the expected rate if creatin were converted into creatinin in the muscle and excreted in the urine. The conclusion is reached that creatinin is formed in muscle from creatin at a constant rate, the creatinin being transported to the kidney by way of the blood and there excreted into the urine.[4]

[1] Page 364.

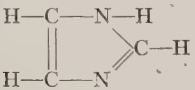
[2] See Myers, cited on page 546.

[3] See page 461. Compare Hammett, F. S. (Fig. 121), Jour. Biol. Chem., vol. 53, p. 323, 1922.

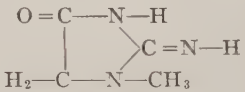
[4] Recently Zwarenstein (page 730) has shown the variation in creatinin excretion over long periods of time (10 to 15 days).

Recently two suggestions relative to the origin of creatinin from creatin have been made. Benedict and Osterberg[1] account for the usual statement that on feeding creatin, only creatin is excreted, by their observations that creatin is retained for days after being fed. Then after the tenth day, creatin began to be excreted and at the same time the creatinin increased in amount until it reached a level 33 per cent. above normal control. Creatinin is only one of every three molecules of creatin metabolized. The site of retention of creatin has not been discovered. Since feeding of creatin causes a lower excretion of total nitrogen, we are compelled to conclude that creatin causes the retention, not only of itself but of other nitrogenous substances.

The second suggestion is that of Zwarenstein[2] who finds a correlation between creatinin and uric acid excretion. A possible explanation of this fact is that in both substances an iminazol ring occurs:



Iminazol.



Creatinin.

Leathes discovered a similar correlation between uric acid and creatinin excretion. If this correlation has a basis of fact indicating an actual interdependence it probably means that both uric acid and creatinin have a common precursor; and since nucleoprotids are the source of uric acid it is assumed by Zwarenstein that the nucleoprotid is also the source of creatinin.

The constancy in creatinin excretion over several hours per day is shown by the following table from Simpson (Two-hour periods):

[1] Benedict, S. R. and Osterberg, E. Studies in creatin and creatinin metabolism. V. The metabolism of creatin. J. Biol. Chem., vol. 56, page 229, 1923. This work has been corroborated by Chanutin, A., Journ. Biol. Chem., vol. 67, page 29, 1926.

[2] Zwarenstein, H. (Victoria University, Manchester, England), Studies in creatin and uric acid metabolism, Biochem. Journ., vol. 20, page 743, 1926.

Time. A. M.	Subject, W. H. J. A., mgs.	Subject, G. E. S., mgs.	Subject, B. P. W., mgs.	Subject, G. W. C., mgs.
8-10	95	..	65	
9-11	..	69	..	78
10-12	84	..	83	
11-1	..	63	..	70
P. M.				
12- 2	86	..	91	
1- 3	..	78	..	66
2- 4	98	..	68	
3- 5	..	68	..	90
4- 6	91	..	76	
5- 7	..	73	..	68
6- 8	80	..	75	
7- 9	..	63	..	61
8-10	95	..	66	
9-11	..	71	..	63
10-12	82	..	76	
Midnight				
11-1	..	72	..	54
12-2	84	..	71	
1-3	..	63	..	63
2-4	78	..	74	
3-5	..	80	..	67
4-6	71	..	75	
5-7	..	65	..	73

Concerning sex differences, it has been found[1] that creatinin is excreted in practically equal amounts by men and women, but in the case of creatin, some apparently normal women excrete a small amount each day.

As to the function of creatin and creatinin in the human body, it has been suggested that the former is concerned in some manner with the muscle tonus. Soldiers standing at "attention" excrete a larger amount of creatinin than when walking, sitting, or lying down. Electrical stimulation of frog's muscles is followed by an increased content of creatin.[2] Biochemists are not in agreement concerning the rôle of these two substances in body fluids and tissues. Creatin has been supposed to:

1. Exist only as a waste-product.
2. Act as food. Creatin is retained to a certain amount when fed.
3. Be intermediate in lecithin formation, suggested by its relation to cholin; or, vice versa, creatin may come from cholin.

[1] McLaughlin, L., and Blunt, K. (University of Chicago, Chicago, Ill.), Jour. Biol. Chem., vol. 58, p. 267, 1923.

[2] See page 734 for discussion concerning the actual presence of creatin in the urine. Benedict (Jour. Biol. Chem., vol. 52, p. 11, 1922) expresses a doubt that the creatin in the blood is anything but a waste-product.

4. Form arginin, suggested by the common possession of the guanidin radicle. Or, vice versa, creatin may be derived from arginin.

5. Have a relation to glucid metabolism. Sugar is necessary for the metabolism of creatin.

6. Maintain muscle "tonus," or to aid in its contraction.[1]

7. Be a detoxicating agent for guanidin, which is associated with tetany.

8. Associated with phosphoric acid in muscle (Fiske).[2]

Clinical Significance of Creatin.—1. In glycopriva. Withdrawing or conspicuously lowering starches and sugars from the diet produces creatinuria. In diabetes mellitus, or in any condition in which the glucid metabolism is interfered with (renal diabetes; alimentary glycosuria; phlorhizin poisoning for experimental purposes or for diagnostic reasons; epinephrin administration, etc.), creatin is excreted.

2. In muscle catabolism, as in poliomyelitis, myositis, dermatomyositis, and in other muscular involvements.

3. Exophthalmic goiter.

4. Fasting; see (1). However, certain protids, like serum protid, and eggs, cause the disappearance of creatinuria. In fact, it is possible that (1) is to be explained by the protid-sparing action of glucids.[3]

5. Excessively high protid diet, especially with certain kinds of protids. This has been offered as an explanation of creatinuria of children. The creatinuria of children and that of exophthalmic goiter cases varies with the time of taking nourishment. Creatinuria can be established in women during intermenstrual periods by high protid feeding. In fact, the expression "creatin diabetes" has been used to signify the creatinuria on high protid diets. The term "diabetes" refers to the elimination of creatin and not of sugar. It is the food protid and not stored protid which gives rise to creatin. In children on a high protid diet creatin reaches a maximum corresponding to the establishment of a positive nitrogen equilibrium.

6. In children creatinuria occurs in both sexes, but the termination of the period during which creatin is excreted is different in the two sexes. It is characteristic of night urine and it may fall under (1) or (5). It persists longer in female children than in males, the former

[1] Looney (Am. J. Physiol., 1924, lxix, 639) shows that the creatin content of the blood varies with the muscular tone.

[2] See page 24.

[3] Page 566.

NITROGEN PARTITION BY GRAMS AND PERCENTAGE FOR NORMAL CHILDREN (JOHNSTON AND VEEDER)

Date and age.	Amount.	Specific gravity.	Total nitrogen.	Ammonia nitrogen.	Urea nitrogen.	Creatinin nitrogen.	Creatin nitrogen.	Uric acid nitrogen.	Per cent. of total nitrogen.					
									Urea.	Ammonia.	Creatinin.	Creatin.	Uric acid.	Undetermined.
"Standard" Diets														
I. 21 months														
10/27–28	750	1.012	4.480	0.173	3.941	0.050	0.037	0.060	87.8	3.9	1.1	0.8	1.3	5.1
10/28–29	550	1.018	4.425	0.176	3.852	0.047	0.027	0.050	87.0	4.0	1.1	0.6	1.1	6.2
Average.....	650	1.015	4.45	0.175	3.897	0.049	0.032	0.055	87.4	3.9	1.1	0.7	1.2	5.7
II. 9 years														
11/ 9–10	440	1.030	7.936	0.354	.....	0.165	0.112	0.069	....	4.5	2.1	1.4	0.9	
11/10–11	410	1.030	8.064	0.325	6.717	0.162	0.098	0.052	83.5	4.0	2.0	1.2	0.6	8.7
Average.....	425	1.030	8.000	0.340	6.717	0.164	0.105	0.060	83.5	4.2	2.0	1.3	0.8	8.2
Creatinin-free Diets														
I. 28 months														
11/29–30	860	1.007	3.968	0.214	3.332	0.049	0.079	0.089	84.0	5.4	1.2	2.0	2.2	5.2
11/30–12/1	860	1.012	3.347	0.259	2.778	0.045	0.064	0.099	83.0	7.7	1.3	1.9	2.9	3.2
12/1–2	705	1.014	3.676	0.213	3.120	0.052	0.074	0.077	85.0	5.8	1.4	2.0	2.1	3.7
Average.....	755	1.011	3.663	0.229	3.077	0.049	0.073	0.088	84.0	6.3	1.3	2.0	2.4	4.0
II. 6 years														
11/29–30	770	1.013	4.975	0.205	4.041	0.081	0.064	0.081	81.0	4.1	1.6	1.3	1.6	10.4
11/30–12/1	1140	1.007	4.800	0.213	3.760	0.100	0.038	0.101	78.3	4.4	2.1	0.8	2.1	12.3
12/1–2	610	1.013	4.733	0.200	.....	0.095	0.024	0.073	....	4.2	2.0	0.5	1.5	
Average.....	840	1.011	4.836	0.201	3.90	0.092	0.042	0.083	80.0	4.2	1.9	0.9	1.7	

terminating creatinuria at puberty, the male children about two or three years earlier.

7. In women there is much variation in creatin excretion. Some women excrete creatin only during catamenia others exhibit intermittent creatinuria. It is supposed that intermittent creatinuria passes over into the continuous creatinuria of pregnancy and other sexual periods. Postpartum urine shows an increased content of creatin over that during pregnancy. It is at its height about the third day after delivery and extends to the end of the first week, owing to the lactation which begins about this time and not to the involution of the uterus, as formerly supposed.

8. In acidosis. Other factors arising during acidosis may be the cause of the creatinuria observed during that condition. It is probable that the cause lies in (1) or in (5) above.

The amount of creatin excretion reported in chemical papers must be considered according to the method used to obtain the figures. It is probable that many of the reports are untrustworthy, owing to the method employed. The table on page 733 represents the work done with accurate modern methods.

The table on page 733 is from Johnston and Veeder.[1]

Folin found that the diet caused a characteristic change in creatin excretion:

Vegetarian diet	8 to 18 mgs. per 100 mls. urine.
Mixed diet	250 (about) " " " " "

In women the average creatin excretion in cases of intermittent creatinuria is less than 0.10 g. During pregnancy it rises to approximately 0.17 g. per twenty-four hours and to 0.42 g. in the first three or four days after delivery. Immediately after delivery there may be a rise as high as 1.5 g., but this is not usual.

Pathologically, creatin appears in increased amounts in:

1. Acute fevers.
2. The later stages of exophthalmic goiter.
3. Diabetes mellitus and interferences with the glucid metabolism.
4. Muscle wastings, like poliomyelitis.
5. "Marasmus"[2] in children.

[1] Johnston, M. R., and Veeder, B. S. (Washington University, St. Louis, Mo.), The Nitrogen Partition in the Urine of Normal Children, Amer. Jour. Dis. Child., vol. 13, p. 404, 1917.

[2] Greek *marasmos*, from *maraino*, drying up. Said of children when they show progressive wastings and emaciation for which there is no obvious cause. The term is non-critical and generally refers to avitaminoses or other nutritional disturbances.

Creatinin, as we have shown above, is a constant excretory product of the urine of both sexes, young as well as adult, although there is a lowered creatinin excretion, which may be correlated with the lower content of muscle creatin (of the newborn 0.07 per cent.; of adult 0.3 per cent.). The creatinin excretion varies with muscle bulk.[1] The average normal twenty-four-hour excretion is 7–11 mgs. per pound of body weight. An adult male weighing 70 kilos[2] excretes about 1.5 gs. in twenty-four hours. Creatinin varies physiologically:

1. With exercise, combined with fasting, the extent varying with the subject.

2. With metabolic rate, especially in women. Smaller, underweight, more active women excrete relatively more creatinin than larger, less active subjects.

3. By feeding meat extracts and other foods containing a high creatin content. Eighty per cent. or more of creatin administered appears as creatinin in the urine after a time. Creatin after feeding is stored as such for a time, but may be "washed out" by copious water drinking.[3]

Pathologically, creatinin is increased in muscle catabolism as in fevers, during the toxic stage, etc. It is diminished in post-pyrexial stages of intoxication, in acute parenchymatous nephritis (nephrosis), in anemias, in tuberculosis, etc. The significance of creatin and creatinin from the standpoint of blood analysis will be discussed later.[4]

Qualitative Test for Creatin.—Principle: Hydrolysis to creatinin. The test is quantitative as follows. (For qualitative tests for creatinin see pages 367 and 368.)

Quantitative Determination of Creatin and Creatinin.[5]—There is no practical method available for the quantitative determination of creatin. The method devised by Folin involves the following principle:

Folin's Method for Creatin: Principle.—Conversion of creatin to creatinin and the estimation of the combined creatinin from creatin and of the "preformed" creatinin existing in the urine. Then, after estimating the latter on a second sample of urine, creatinin from creatin is obtained by subtraction.

[1] But not with muscle exercise. See (1) concerning exercise, combined with starvation.

[2] 154 pounds.

[3] Fowler, C. C. and Hawk, P. B., Journ. Exper. Med., vol. 12, page 388, 1910.

[4] Chapter XVI.

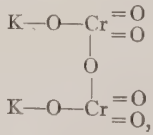
[5] Page 369.

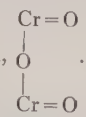
Procedure: Pipette 10 mls.[1] of urine into a small Erlenmeyer flask. Add 10 mls. of 10 per cent. hydrochloric acid. Place the flask without stopper in an autoclave and leave for ten minutes at a temperature of about 115° C. Remove, let cool, and transfer to a 500-ml. volumetric flask. Add 10 per cent. NaOH to neutralization and then 5 mls. more. Add 15 mls. of saturated picric acid solution and let stand just five minutes. Dilute to the mark and compare with one of the two following standards:

1. A half-normal solution of potassium dichromate[2] made by weighing out 24.10 gs. pure potassium dichromate[3] and making up to 1 liter with H_2O. However, the color of the dichromate solution is qualitatively different from that of the red creatinin picrate produced in this determination. Moreover, in calculating, compensation must be made for the slight quantitative difference between the color of the solution of creatinin picrate that corresponds to a standard solution of creatinin and the half-normal dichromate solution. The basis of the calculation is as follows: Ten milligrams of creatinin treated with picric acid and alkali as described above and made up

[1] If desired, 1.0 ml. urine may be used. In this case, use a 100 ml. volumetric flask, 20 mls. of saturated picric and 1.5 mls. NaOH 10 per cent. The flask may be stoppered with tin-foil.

[2] Potassium dichromate, $K_2Cr_2O_7$, may be given the following structural formula,



in which chromium is hexavalent. To determine the normal value for making a standard solution we find the change in valency when the dichromate is used to oxidize a substance. On being reduced chromium becomes trivalent, since the dichromate is reduced to chromic oxid,  . Hence, since a gram-molecule made up to a liter is a normal when expressed in gram equivalent and since the gram equivalent is defined as the weight in grams corresponding to one unit of valence change, we have for each chromium atom a valence change of 3; that is, $6 - 3 = 3$. Therefore, to make a normal solution of $K_2Cr_2O_7$ we must take one-sixth of the molecular weight of the dichromate. Mol. wt. $K_2Cr_2O_7 = 290.20$; divided by 6, we have 48.36. Hence, 48.26 g. $K_2Cr_2O_7$ dissolved and made up to 1000 mls. of solution in water make a normal solution, and a half-normal solution is $\frac{48.36}{2} = 24.10$ gs. $K_2Cr_2O_7$ made up to 1000 mls. with water.

[3] Since the word is of Greek origin (Greek *chromos*, color) the Greek prefix *di* (Greek *dis*, two) must be used rather than the Latin *bi-* (Latin *bis*, two).

to 500 mls. of solution give a color slightly less pronounced than that of a half-normal dichromate solution; with the dichromate at 8 mms. and the creatinin at 8.1 mms., the colors match. The standard, therefore, is set at 8, but since the standard dichromate solution simply replaces the standard creatinin solution, in calculations, 8.1 is the standard reading. Hence, with dichromate as standard:

$$\frac{\text{Reading of the standard} = 8.1}{\text{Reading of the unknown} = n} = \frac{\text{Concentration of unknown (x)}}{\text{Concentration of standard (10 mgs.)}}.$$

Solving for x, we have:

$$\text{Creatinin in 10 mls. urine} = \frac{81}{\text{Reading of the unknown}}.$$

2. Creatinin standard: To make the standard for colorimetric reading pipette 1 ml. of the standard creatinin solution[1] containing 1 mg. of creatinin into a 100-ml. volumetric flask and add 20 mls. saturated picric acid solution and, lastly, 1.5 ml. of 10 per cent. NaOH delivered from a burette. Let stand five minutes, dilute to the mark, and read in the colorimeter. The calculation is simple: Apply the colorimetric formula used previously in (1) above.

The amount of creatin is then found by making a determination of the preformed creatinin by repeating the foregoing method, but omitting the hydrolysis with hydrochloric acid and subtracting the figure obtained from that derived after hydrolysis.

Modifications of foregoing method have been made by Benedict:

1. Hydrolysis is accomplished in an open evaporating dish. Add a pinch of powdered lead to prevent discoloration of the fluid. Boil rapidly over a free flame until a syrup is obtained and continue the evaporation on a steam-bath. The remainder of the procedure is like that already given.

2. A picric acid-sodium hydroxid solution is made up together, to be used in place of the separate solutions. The mixing must be done immediately preceding the use of the solution.

3. A specially purified picric acid should be used in place of the ordinary commercial kind. Directions for purifying the commercial picric acid are given in the Appendix.

Folin's Alternative Method for Creatin.—Pipette, by means of the Ostwald-Folin pipette exactly 1.0 ml. of urine into a large Erlenmeyer flask (capacity about 300 mls.). Add 20 mls. of saturated picric acid solution.[2]

[1] One gram of creatinin or 1.61 gs. creatin-zinc-chlorid dissolved in one liter of 0.1 normal HCl.

[2] This solution is pure picric acid dissolved in distilled water to saturation.

On the laboratory scales, weigh flask and contents. Now add an excess of 150 mls. distilled water. Place the flask on an asbestos-gauze over a free flame and concentrate the fluid within the flask until the original weight is obtained approximately. Cool. Add 1.5 mls. 10 per cent. NaOH solution. Leave about 10 minutes and transfer the contents to a 100 ml. volumetric flask. Rinse, bringing the volume to the mark. Mix the contents of the volumetric flask and read in a colorimeter against a standard. This determination gives the combined preformed creatinin and that derived from creatin.

Fig. 222.—Meker type Bunsen burner used for ashing residues in analysis of mineral substances, etc.

The *determination of creatinin* in the urine is made by the method given above for creatin, omitting the hydrolysis with acid. The creatinin of urine is spoken of as "preformed" creatinin.[1] The following method of Folin involves the use of 1 ml. of urine:

Folin's Method for Creatinin in the Urine.—Transfer by means of a 1-ml. Ostwald-Folin pipette[2] 1 ml. of urine to a 100-ml. volumetric flask. Wash the pipette with a stream of distilled water and drain as much water as possible, blowing the excess upon filter-paper. Transfer 1 ml. of the standard creatinin solution (mentioned on page 737) to a second 100-ml. volumetric flask. Add to each flask 20 mls. of saturated picric acid solution,[3] and from a burette, 1.5 mls. of 10 per cent. sodium hydroxid solution. Leave ten minutes. Dilute to the mark with distilled water and mix. Read against one another in the colorimeter. In this case the standard may be set at any height, since pure creatinin[4] is used as a standard. Calculation: The calculation is similar to that of the foregoing method, the concentration of the standard being 1 mg.

[1] See page 367.

[2] For the use of these pipettes see the Appendix. As Benedict, Greenwald, and others insist, urines containing "aceton" substances must first be boiled in order to remove such compounds, for they react with the reagent picric acid similarly to creatinin (see page 739). See Blau, N. F. (Cornell Medical, New York, N. Y.), Jour. Biol. Chem., vol. 48, p. 105, 1921.

[3] This solution must be made from purified picric acid, mentioned above, owing to the small amounts of urine and reagents used.

[4] Or pure creatinin-$ZnCl_2$.

Qualitative Tests for Creatinin. **Jaffe's Test** (see page 367).—The test follows the quantitative method for preformed creatinin just given. Place 5 mls. of urine in a test-tube and add 1 ml. of saturated solution of picric acid and also 5 drops of 10 per cent. NaOH solution. Shake the tube. The color gradually becomes reddish, owing to the formation of a red creatinin picrate.[1] Add 1 drop of glacial acetic acid; if done within ten minutes, the color becomes yellow by the re-formation of picric acid. If aceton is present, the color will become reddish brown. Glucose requires long exposure or heat to produce a somewhat similar color, due to picramic acid.[2]

Weyl's[3] Test.—Place 5 mls. of urine in a test-tube and add 5 drops of a freshly made 5 per cent. solution of sodium nitroferrocyanid (nitroprussid) and a few drops of sodium hydroxid solution, made by diluting 10 per cent. solution one-half. Note a permanganate color. Add 1 drop of glacial acetic acid and boil; the color turns greenish and blue, owing to the Prussian blue formed in the reaction. Note: In order to distinguish creatinin from aceton,[4] which gives somewhat similar color reaction, observe that on the addition of the acid in the nitroprussid test for creatinin, the color is at first greenish and later may turn blue if creatinin is present, whereas aceton, treated in like manner, gives a purplish-red color.

Hippuric Acid.—This conjugated amino-acid[5] is found in varying amounts in human urine, ranging from 0.8 to 1.9 g. per twenty-four hours. The amount excreted increases when the glycin content of the body is raised, but experimentation tends to show that increasing the benzoic acid in the body does not cause accelerated excretion of hippuric acid.[6] Hippuric acid is increased only when protids containing the amino-acid glycin or substances capable of being converted into glycin, such as alanin, are administered. Hippuric acid is increased and urea is decreased when benzoates are administered;[7]

[1] Greenwald, I., and Gross, J., Jour. Biol. Chem., vol. 59, p. 601, 1924.

[2] The substances used in the above test are similar to those used in the quantitative method for glucose by Benedict. With glucose, picramic acid is formed by reduction (page 163). Besides creatinin sodium picrate, rubidium, and cesium creatinin picrate have been synthesized (Greenwald and Gross, cited above).

[3] Weyl, T., German chemist, died 1913. Compare page 368.

[4] Page 531.

[5] Page 243.

[6] Lewis, H. B., and Griffith, W. H. (Universities of Michigan and Illinois, respectively, Fig. 102), Jour. Biol. Chem., vol. 55, p. 22, 1923.

[7] Swanson, W. W. (page 741), The Effect of Sodium Benzoate Ingestion on the Composition of the Blood and Urine, with Especial Reference to the Possible Synthesis of Glycine in the Body, Jour. Biol. Chem., vol. 62, p. 565, 1925.

glycin is synthesized from the nitrogenous substances in the body which would otherwise form urinary urea and be excreted as such.

Hippuric acid is increased in cases of intestinal stasis in which there is extensive putrefaction; in diseases of the liver and in acute intoxications with high fever. A distinction must be made between the production and the elimination of this and other urinary constituents. Hippuric acid is absent from the urine or occurs in but small quantities in the retention of nephritis and in hypoplasia of the kidneys.

There is no specific test for hippuric acid other than crystallization.[1] Crystals are obtained from the urine by the following method: Place 200 mls. of freshly voided urine in an evaporating dish and boil over a free flame until the contents have been reduced to a syrup. Transfer the residue to a small Erlenmeyer flask. Wash the last traces into the flask from the dish, using a small amount of water. Add 2 mls. of concentrated sulphuric acid and stopper. Let the preparation remain overnight, or until the following period. Then filter through dry paper and collect the precipitate, which is hippuric acid mixed with uric acid and other nitrogenous and mineral substances. Lay the paper upon the desk to dry and then wash off the crystals into a small Erlenmeyer flask by means of a few mls. of acetic ethyl ester.[2] Add more of the ester and shake in order to cause the extraction of the hippuric acid. Decant the liquid from the residue into a small beaker and let it evaporate. When crystals appear examine the preparation under the microscope.

Quantitative Determination of Hippuric Acid (Folin).—Principle: Hippuric acid is hydrolyzed to glycin and benzoic acid. The acid is extracted with chloroform and titrated with alkali (standard solution of sodium ethoxide, or "alcoholate"). Procedure: Pipette 100 mls. of urine into an evaporating dish and add 10 mls. of 5 per cent. NaOH solution. Evaporate to dryness on a steam-bath. Using not over 25 mls. of half-concentrated nitric acid,[3] dissolve the residue, working it up by means of a "policeman" and then transfer the solution and residue to a 500-ml. Kjeldahl flask, provided either with a reflux condenser or a long glass tube to serve as such. Add a small crystal of cupric nitrate and some lump pumice to prevent bumping

[1] Hippuric acid, on heating in a dry test-tube, gives off the odor of benzaldhyde, or oil of bitter almonds, but this is not distinctive.

[2] Or "acetic ether," also called ethyl acetate, $CH_3.COO.C_2H_5$, a solution of 90 or 99 parts of the ester in water.

[3] The desk reagent HNO_3 diluted with an equal part of water.

and boil the mixture for not less than five hours over a microburner. Withdraw the flame and let the flask cool. Wash the long tube or the condenser with a jet of water from your wash-bottle and transfer the contents of the flask to a separatory funnel. Use about 25 mls. of water in making the transfer. You should have by this time about 100 mls. of solution in the funnel. Now add to the funnel 55 gs. of crystalline ammonium sulphate and let them dissolve. Then add 50 mls. of chloroform and mix.[1] Open the stopper, and then the cock, carefully, and drain off the lower chloroform layer into a flask. Close the stop-cock, add 35 mls. of fresh chloroform and repeat the extraction. Repeat with two 25-ml. volumes of chloroform and transfer the combined extracts to a large separatory funnel. Add 100 mls. of a special sodium chlorid-hydrochloric solution,[2] shake the vessel thoroughly, and then, after the zones separate, draw off the chloroform layer below into a flask. To the flask add a few drops of phenolphthalein (indicator). Titrate with standard sodium ethoxid solutions.[3]

Fig. 223.—Squibb separatory funnel.

Calculation: The molecular weight of hippuric acid is 179.1. A normal solution contains that number of grams per liter, each ml. of which contains a thousandth of 179.1 gs., or 0.1791 g. The sodium ethoxid is decinormal and the results will be in terms of decinormality. Since all decinormals are volumetrically equivalent, the hippuric acid will be decinormal and each ml. will contain 0.01791g. of hippuric acid. One hundred mls. will contain a hundred times that figure, or 1.791 g. In order to convert from volume of hippuric acid to mass multiply each ml. of the titration by 1.791, which gives the per cent. hippuric acid.

Method of Kingsbury and Swanson.[4]—Principle: This method differs from the Folin method in its earlier part, the speed of hydroly-

[1] It is necessary to invert the funnel and to release the compressed gas formed by cautiously opening the stop-cock. It is easier to do it in this manner than to unstopper the upper aperture.

[2] Four hundred grams of solid NaCl are dissolved in 900 mls. of water and 0.5 ml. of concentrated HCl is added. Make up to 1000 mls. with distilled water.

[3] Appendix.

[4] Kingsbury, F. B. (Biochemist, Metropolitan Life Insurance Co., New York, N. Y.), and Swanson, W. W. (University of Minnesota), A Rapid Method for the Determination of Hippuric Acid in Urine, Jour. Biol. Chem., vol. 48, p. 13, 1921.

sis being increased by using solid NaOH and magnesium oxid. The urinary pigments are oxidized with potassium permanganate.

Procedure: Place 50 mls. of urine in a large Kjeldahl flask and add 7.5 gs. of sodium hydroxid and 0.5 g. magnesium oxid. Boil over a free flame, so that at the end of thirty minutes the volume has been reduced to about one-half. Now add 1 ml. of a 7 per cent. $KMnO_4$ solution.[1] Wash down the last trace of permanganate by means of a jet of water from your wash-bottle, using as little water as possible. Now twirl the flask for about one minute, cool under the tap, and add 30 mls. concentrated nitric acid, pouring the acid down the inside of the neck of the flask. Stopper with a cork bearing a condenser[2] and boil for three-quarters of an hour. Cool. Rinse off the condenser, transfer the liquid to a small separatory funnel, and add 25 gs. crystalline ammonium sulphate. When this is dissolved add 50 mls. of chloroform,[3] extract, draw off the chloroform and repeat with 35 mls. and then with 25 mls. volumes. Combine the extracts in a large separatory funnel and add the NaCl-HCl solution given above for the Folin procedure. Draw off the chloroform layer through a funnel provided with dry paper into an Erlenmeyer flask. Add a few drops of phenolphthalein indicator and titrate with sodium ethoxid decinormal solution to a definite pink color.

Calculation: The number of mls. of decinormal sodium ethoxid solution used times 1.791[4] gives the number of grams of hippuric acid per 50 mls. of urine.

Clinical Interest in Hippuric Acid.—The rate of excretion of hippuric acid has been utilized as indicative of liver function[5] and renal efficiency.[6] The former depends upon the synthesizing power of the liver for hippuric acid, the latter the power of the kidney to excrete hippuric acid. For diagnosis of hepatic inefficiency the hippuric acid is determined in the urine on a standard diet, preferably for two or three days previous to the test. Then a given amount of sodium benzoate is given by mouth and the same day the hippuric acid in the urine is determined. If there is an increase in hippuric acid excretion,

[1] This is meant to be a saturated solution at the ordinary room temperature (20° C.).

[2] Compare the Folin procedure, page 740.

[3] For Kingsbury's method of purifying commercial chloroform see Appendix.

[4] Page 741.

[5] Lackner, E., Levinson, A., and Morse, W. (Michael Reese Hospital, Chicago), Biochem. Jour., vol. 12, p. 184, 1918.

[6] Kingsbury and Swanson (note 1, above), Arch. Int. Med., vol. 28, p. 220, 1921. Morgulis, S., Pratt, G. P., and Jahr, H. M. (University of Nebraska College of Medicine, Omaha), Arch. Int. Med., vol. 31, p. 116, 1923.

it is indicative of normal hepatic function; failure of increase or any increase below the theoretical normal amount computed from the ingested benzoate indicates that the liver is not functioning properly. Concomitant with the estimation of hippuric acid, urinary chlorids are determined in order to indicate whether the kidney is functioning normally. In the renal test the bladder is emptied and 2.4 gs. of sodium benzoate dissolved in about 100 mls. of water are administered by mouth at any time during the day. Fruit is eliminated from the diet. At the end of three hours urine is voided, or that voided during the three hours is collected and made up to 500 mls. volume, of which 100 mls. are taken for analysis. The result is expressed in terms of per cent. of ingested benzoate. In cases of oliguria, it may be necessary to extend the period of collection of urine over a longer time. The following table from Kingsbury and Swanson shows the results for selected cases:

Class.	Hippuric acid excretion. Per cent. of ingested C_6H_5COONa excreted.
Normals	56
	96
	104
	52
	73
Chronic nephritis	24
	59
	28
	53
	81
Cardiac decompensation	26
	51
Mitral stenosis	66
	103
Chronic interstitial nephritis	45
	94

Morgulis gives figures for cases of similar nature for six-hour periods.

Amino-acids.—In normal human urine amino-acids are excreted daily, the amount varying with the individual and with the character of the food. The normal excretion averages about 4 to 12 mgs. per hour. It is possible that about 1 gram of glycin, besides that conjugated as hippuric acid, is excreted daily. No direct correlation between physiological states and amino-acid excretion has been made. Pathologically, amino-acids are found excreted in large amounts in almost any condition in which the liver function is involved. This probably does not indicate an interference with the deaminizing power of the liver, which is shared with all organs, and especially, as

Folin has demonstrated, with the muscles; but rather, as Van Slyke's observations have shown, that the normal liver absorbs large amounts of amino-acids and a deranged liver throws into the circulation excess amounts of amino-acids, with consequent excess amounts for excretion through the kidneys. In conditions in which there is a large and sudden production of amino-acids, as in the resolution of the exudate in pneumonia through autolysis, amino-acids appear in the urine in increased quantities.

Tests for the presence of amino-acids in the urine follow the same procedure as those for amino-acids in digests,[1] etc. The "Ninhydrin" test is available, but it must be recalled that this test gives other dialyzable protidtemns than amino-acids.

Quantitative Determination of Amino-acids in the Urine (Method of Folin).—Principle: The ammonia of the urine is removed by permutit[2] and the solution of amino-acids remaining is treated with a quinon compound which changes color, quantitatively, in the presence of amino-acids. The color is compared in a colorimeter with that developed by a similar treatment of known quantities of pure amino-acids, like glycin.

For reagent see Appendix.

Procedure: Secure four test-tubes which have been graduated at 25 mls. and to each of three of these tubes add, respectively, 1, 2, and 3 mls. of the standard amino-acid solution. To each tube add similar amounts, respectively, of the special 1 per cent. sodium carbonate solution. Add water to each tube to make a volume of 10 mls. Now prepare the specimen of urine: Place 25 mls. of urine in a 50-ml. Erlenmeyer flask and dilute to 50 mls. Add about 2 gs. of permutit powder and agitate the contents of the flask for not less than five minutes. Let the powder settle and from it decant the supernatant liquid into another flask, draining the permutit as dry as possible. Repeat this procedure once, agitating the contents of the flask for not less than five minutes. Then pipette 5 mls. of this ammonia-free urine into the fourth test-tube. Add 1 ml. of decinormal HCl solution and 1 ml. of the special 1 per cent. Na_2CO_3 solution. Dilute as with the standards, to 10 mls.

Prepare the reagent:[3] Weigh out 250 mgs. of quinon and dissolve the powder in 50 mls. distilled water. Then to each of the four tubes add 5 mls. of the quinon solutin. Mix. Place in a dark closet overnight, or until the following period. Then, to each tube add 1 ml. of the special acetic acid-acetate solution and 5 mls. of 4 per cent.

[1] Page 239. [2] Page 715. [3] Appendix.

sodium thiosulphate solution, which destroys the surplus quinon. Dilute each tube to the mark (25 mls.). Compare the unknown with the standard which it resembles most closely (1, 2, or 3). The comparison must be made within one hour, because after that the thiosulphate attacks the compounds responsible for the color produced by the amino-acids acting upon the quinon.

Calculation: Each ml. of the standard amino-acid solution contains 0.07 mg. of amino-acid nitrogen. Standard Number 1 contains 0.07 mg., standard Number 2, 0.14 mg. and Number 3, 0.21 mg. of nitrogen. Assuming that the calculation is being made for Number 2:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown (n)}} = \frac{\text{Concentration of unknown (x)}}{\text{Concentration of standard (0.14)}}.$$

If the standard is placed at 20, then:

$$x = \frac{2.8}{n} \text{ mgs. amino-nitrogen per 5 mls. of 50 per cent. urine.}$$

Multiply the result by 40[1] to give percentage of NH_2 nitrogen in the urine.

Method of Van Slyke for Total[2] *Amino-acids in the Urine.*—Principle: The amino-compounds are hydrolyzed by means of acid, the time of hydrolysis being hastened by autoclaving. The neutralized and concentrated residual fluid is then analyzed in the Van Slyke apparatus[3] by gasometric determination of amino-nitrogen.

Procedure: Pipette 25 mls. of urine into a 50-ml. volumetric flask and add 1 ml. of concentrated sulphuric acid. Cover the mouth of the flask with tin-foil, leaving minute openings for the equilibration of the gases. Place the flask in an autoclave or oil-bath at 180° C. and leave for 1.5 hour. At the end of the period add 2 gs. powdered calcium hydroxid, agitate the contents, and then dilute to the mark. Filter, preferably with a piece of folded filter (Fig. 218). Transfer 20 mls. of the filtrate to an evaporating dish and leave on the steam-bath until dry. Take up the residue in 1 ml. of 50 per cent. acetic acid solution, afterwards diluted with not more than 5 mls. of water. Use a rubber-tipped "policeman" to scrub the dish. Pour the liquid into a 10-ml. volumetric flask. With a few drops of water dilute to 10 mls. and use 2 ml. amounts of the liquid for the determinations in the smaller apparatus or two aliquots of 5 mls. each for

[1] The urine was diluted one-half (50 per cent.); 5 mls. of this diluted urine were taken. To give 100 mls. multiply by 20, and for full strength, multiply by 2; hence $2 \times 20 = 40$.

[2] That is, the conjugated amino-acids, like hippuric acid as well as the uncombined.

[3] Page 294.

the larger apparatus. The following guide to the time of deaminization should be read:

From: 15° to 20° C.	4 to 5 minutes
20° to 25° C.	3 minutes
25° to 30° C.	2.5 minutes

Calculation: See the description of the method on page 298.

Free or Preformed Amino-acid in the Urine (Method of Van Slyke).—Principle: Urea is decomposed by urease[1] and the ammonia is neutralized. The filtered solution is then analyzed by the gasometric method given above.

Procedure: Pipette 25 mls. of urine into a 50-ml. volumetric flask and add 2 mls. of urease solution.[2] Leave at 50° C. for two hours, or overnight at 37° C. Then add 10 mls. of a 10 per cent. suspension of calcium hydroxid and make up to the volume (50 mls.). Filter. Evaporate as in the preceding method for total amino-nitrogen and proceed from this point according to that method.

The calculation is similar to that of the foregoing method. The result gives the uncombined amino-nitrogen.

Rapid Clinical Method of Henriques and Sørensen.[3]—Principle: Methylation of the amino-radicle of the amino-acids, thus destroying the basic properties of this radicle and permitting the carboxyl acidity to manifest itself. The principle is essentially that of the determination of ammonia by the formol-titration method.[4] A preliminary treatment of the urine to remove ammonia, phosphates, etc., is necessary.

Reagents: These are similar to those of the method referred to above.

Procedure: Pipette 50 mls. of the urine into a 100 ml. volumetric flask and add 1 ml. phenolphthalein (indicator). Add 2 gs. barium chlorid solid. Agitate the contents of the flask so that the fluid will become saturated with the barium salt. Now add a saturated solution of barium hydroxid until the solution turns pinkish; this is due to the phenolphthalein in the alkaline solution. Add an excess of 5 mls. of the hydroxid. Fill to the mark, stopper, and invert. Let stand fifteen minutes and then filter. Measure 80 mls. of the filtrate into a second 100-ml. volumetric flask and neutralize the liquid (litmus-paper). Make up to the mark with freshly distilled water. Proceed

[1] Page 113. [2] Appendix.

[3] Henriques, V. (Professor, Copenhagen), and Sørensen, S. P. L. (Carlsberg Laboratory, same institution), Zeitschr. f. physiol. Chemie, vol. 64, p. 120, 1909.

[4] Pages 291 and 718.

to the titration: Pipette 20 mls. of the fluid into a beaker and add 0.2 normal sodium hydroxid solution until the color is pinkish. Then add 10 mls. of the neutralized formalin and carefully titrate to the same pink color. In order to fix this color in mind make a control of 20 mls. of distilled water, to which 20 mls. of the formalin solution is added. Add a few drops of phenolphthalein solution and titrate with the alkali to the first trace of pink color. Add 1 drop of the alkali; the color deepens. Add 2 drops more and the deep reddish pink color used for color comparison will be obtained. Bring the unknown to this color by adding alkali. Note amount of alkali used from burette.

Calculation: The calculation resembles that for Kjeldahl determinations of total nitrogen. The alkali being twice decinormal (0.2 normal), each ml. corresponds to 2×0.0014 g., or 0.0028 g. nitrogen. This factor multiplied by the number of mls. used from the burette gives the number of grams of amino-nitrogen derived from the amino-acids in 20 mls. of the diluted filtrate, which was obtained by diluting 80 mls. of the original filtrate to 100 mls. of solution. Then five times the result gives the amino-nitrogen in 80 mls. of the original filtrate, and since this filtrate was 50 per cent. pure urine, the 80 mls. correspond to 40 mls. of pure urine. To express in per cent., multiply by 2.5; that is, $\frac{1}{40} \times 100 = 2.5$.

Undetermined Nitrogen.[1]—The character of about 4 per cent. of the nitrogen of the urine is still undetermined. After the urea, uric acid, ammonia, and other known forms of nitrogen have been determined, this fraction remains. It seems to vary with the diet and has not been correlated with any special physiological or pathological factor other than conspicuous changes of diet.

"*Colloidal Nitrogen.*"—This term was introduced by Salkowski[2] in 1905 to designate that alcohol-precipitable nitrogen which does not dialyze (hence the term). Normally, it constitutes about 2 per cent. of the total nitrogen, but in cases of malignancy may become increased even to 10 per cent. of the total nitrogen. This increase is not, however, invariable and is not particularly reliable in clinical diagnosis, for other conditions, such as hepatic cirrhosis, also lead to a similar increase. Some of the substances making up colloidal nitrogen contain sulphur, and there has been a correlation established between the amount of sulphur and colloidal nitrogen, on one hand, and the rate of growth of carcinomas, on the other.

[1] See Jour. Amer. Med. Assoc., vol. 80, p. 1007, 1923. [2] Page 216.

It is probable that the undetermined nitrogen is composed of simple amino-acids, while the "colloidal nitrogen" is either mixed with urea or a compound from which urea may be derived by comparatively simple process. Such seems to be the character of the "alloxyproteïc" and the "oxyproteïc" acids which have been described as occurring in the "colloidal" or undetermined fraction of the urine.

Allantoin[1] occurs normally in the urine, even in the case of purin-free diet, in amounts to from 0.012 to 0.014 g.[2] The substance has already been mentioned in connection with uric acid[3] as an oxidation product. Liebig determined that if uric acid was moderately oxidized, allantoin was obtained and that with more prolonged oxidation, other products occurred. From the following evidence it has been assumed that allantoin is the result of the destruction of uric acid by oxidation in the body:

1. When uric acid is ingested allantoin appears in the urine.

2. When a gland substance, like thymus, rich in purins, is fed, allantoin appears in larger amounts in the urine, but when purin-poor materials, like muscle, are fed, the allantoin is recovered only in small amounts, as on a purin-free diet.

3. When allantoin is fed to the dog, which excretes relatively considerable amounts of allantoin, 70 per cent. appears in the urine, but when fed to man, only 20 per cent. is recovered from the urine.

4. In a large series of animals,[4] the uric acid and allantoin excretions are in inverse proportion.

5. Uric acid is destroyed in a digest of tissues from organisms which normally excrete allantoin, but not in digestions of human tissues. However, the following points argue against the theory that allantoin represents the degree of destruction of uric acid:

1. Allantoin is excreted uniformly on a purin-free diet.

2. There is no increase in cases of leukemia and of gout when a purin-free diet is fed.

3. Following subcutaneous injection of uric acid into dogs in certain carefully controlled cases, about half of the uric acid was excreted and very little allantoin could be discovered.

[1] So called because of its presence in the allantoïc fluid in the allantois, or primitive urinary bladder (Greek *allas*, sac, like sausage, and *eidos*, like).

[2] Wiechowski, W., Biochem. Zeitschr., vol. 25, p. 431, 1910.

[3] Pages 338 and 373; allantoin precedes alloxan in oxidation; see also Chapter XVI concerning blood uric acid.

[4] See Hunter's series (Hunter, A., and Ward, F. W., Toronto), Trans. Roy. Soc., Canada, vol. 13, series iii, sec. v, p. 7, 1919.

4. The "uricolytic index"[1] of the dog is about that of the goat, yet when the goat ingests a known amount of uric acid, there is a dissimilar concentration of uric acid in the blood after a given time. One and a half hours after injection there remains in the blood of:

	G. uric acid per 100 mls. blood.
Dog	0.0020
Goat	0.0095

The goat destroys less uric acid than the dog.

The clinical interest in allantoin is slight, principally because so little is known regarding the origin of allantoin and its relations to the uric acid of the urine. There are inherent errors in the methods of determination of very small amounts of allantoin in the fluids of the body; these errors frequently overbalance the slight variations indicative of disease. Allantoin has been reported to be increased during pregnancy, but it must be recalled that it is a normal secretion of the urine of the fetus. Moreover, nurslings show relatively and actually larger amounts of urinary allantoin than adults.

Chemical Characteristics.—In tepid water allantoin crystallizes into glistening white prisms (m.-p., 231° C.). Dissolved in hot water or in ethanol, they give a neutral reaction. Ten per cent. sodium hydroxid solution in the cold dissolves the crystals. No odor or taste is imparted by the aqueous solution or during decomposition. Like purins in general, allantoin is precipitated by ammoniacal silver nitrate and silver lactate solutions. There is no qualitative test. The allantoin silver salt may be distinguished by determining the content of silver, which is 40.73 per cent. by weight. When the crystals of allantoin are decomposed by boiling alkali, the solution treated with a reagent containing an indol substance, and the solution layered with concentrated sulphuric acid the glyoxalic reaction[2] is obtained.

Qualitative Test.—Dissolve, by gentle heat, one crystal of allantoin in 5 mls. distilled water. Add one scale of indol. Mix. Underlay the cooled preparation with concentrated H_2SO_4. A purple ring indicates the presence of allantoin, which has been converted into glyoxalic acid, which, in its turn, reacts with indol exactly as it does in the Hopkins-Cole reaction for tryptophan (page 312).

[1] Percentage of uric acid oxidized to allantoin, estimated by the ratio:

$$\frac{\text{Per cent. allantoin}}{\text{Per cent. uric acid} + \text{allantoin}}.$$

[2] Page 312.

Quantitative Method of Folin.[1] Principle: Allantoin, along with the urea of the urine, is converted to ammonium carbonate[2] by acid treatment, and in another procedure the urea is determined by the specific action of urease. Allantoin is then obtained by difference.

Procedure: Place 5 mls. of urine in a 200-ml. Erlenmeyer flask, fitted with a reflux condenser. Add 20 gs. of magnesium chlorid and 5 mls. of concentrated hydrochloric acid. Add an indicator, like methyl red, and 5 mls. of paraffin oil to reduce surface tension and prevent frothing. Apply the heat so that the condensed fluid falls in drops, three or four to the minute. Boil for an hour, tilting the flask and condenser if the urea tube is used,[3] in order that the liquid in the flask may remain acid in reaction. Discontinue heating at the end of the hour and transfer the contents of the flask with a little wash water to a 700-ml. Kjeldahl flask, or a "balloon" type flask (Fig. 262), attached to a Kjeldahl or Liebig condenser. The procedure of the Kjeldahl method for total[4] nitrogen is followed from this point. Add not over[5] 10 mls. of 40 per cent. NaOH solution, pouring the alkali down the inside of the neck of the flask and permitting it to layer. After the decinormal acid in the milk bottle below the condenser has been brought into communication with the lower outlet of the condenser, attach the flask to the distilling apparatus and then agitate the contents of the vessel until they are thoroughly mixed. The reaction must be alkaline. Distill for about one hour. Titrate the excess of decinormal acid in the milk bottle as in the Kjeldahl method for total nitrogen.

Calculations: The titration gives the amount of acid left after all the ammonia has been neutralized and the figure is in terms of decinormality. Therefore, to convert the volume of ammonia nitrogen indicated by the titration figure to mass of nitrogen, multiply the titration mls. by the weight of nitrogen in 1 ml. of decinormal solution of nitrogen, 0.0014 g. The result gives the combined nitrogen derived from allantoin and urea in 5 mls. of urine.

Second procedure: Make a determination by one of the urease methods given on pages 710–712 of the urea content of the same urine and calculate for 5 mls.

[1] Modified by Plimmer, R. H. A. (page 220), Biochem. Jour., vol. 8, p. 641, 1914.

[2] For the method of decomposition see page 710.

[3] If a reflux condenser is used, a few drops of concentrated hydrochloric acid are added.

[4] Page 287.

[5] Magnesium hydroxid is formed and is precipitated, which causes bumping.

Final calculations: Subtract the second from the first result; the figure obtained represents the grams of allantoin nitrogen in 5 mls. of urine.

Christman[1] has proposed a method for quantitative determination of allantoin by causing it to be hydrolyzed by alkali to oxalic acid, the oxalic acid then being determined as calcium oxalate, which, in turn is quantitatively analyzed by permanganate titration.

The nitrogenous substances just discussed comprise all those which are derived from the normal metabolism of the body, but certain nitrogen-bearing substances of adventitious nature also occur, which are derived from secretions, bacterial decompositions, etc.

Mucin.—This substance is derived from the urinary ducts and vessels, the walls of which, like mucous membranes in general, secrete certain substances, including the mucoprotid, mucin. Like the mucin of the saliva and of connective tissue, it is precipitated by adding dilute acid. Mucin may be confused with albumin, but the two are readily differentiated: Albumin is precipitated by acid, and unless a large excess of acid is used it remains precipitated. On the other hand, mucin is precipitated by dilute acid and dissolves in excess. The nature of urinary mucin is not entirely clear. Besides the acid-precipitatable substance, it occurs in the "nubecula"[2] in an insoluble state. Mucin is increased in catarrhal conditions of the urinary tract. Other substances differing from mucin are precipitated from the urine by adding acid, the nucleo-albumin being an example. It may be distinguished from mucin by its insolubility in moderately concentrated acid. The term is doubtless a misnomer, because the substance contains no phosphorus, as the so-called nucleo-albumins such as vitellin and casein do. The presence of chondroiten sulphuric acid suggests that the so-called "nucleo-albumin" of urine belongs rather to the scleroprotids.[3]

The test for mucin is the same as that for other forms of mucin; it is a glucoprotid and, when boiled with acid, is hydrolyzed. The glucose is split from the protid and may be detected by making a Benedict sugar test.[4]

Urinary Diastase.—A substance capable of hydrolyzing starch was discovered in 1863 by Conheim in the urine. It is present to the greatest extent before breakfast. Injury to the kidney causes a marked diminution in the amount of this enzyme in the urine. It is

[1] Christman, A. A. (University of Michigan), Journ. Biol. Chem., vol. 70, page 173. 1926.

[2] Page 685. [3] Page 303. [4] Page 160.

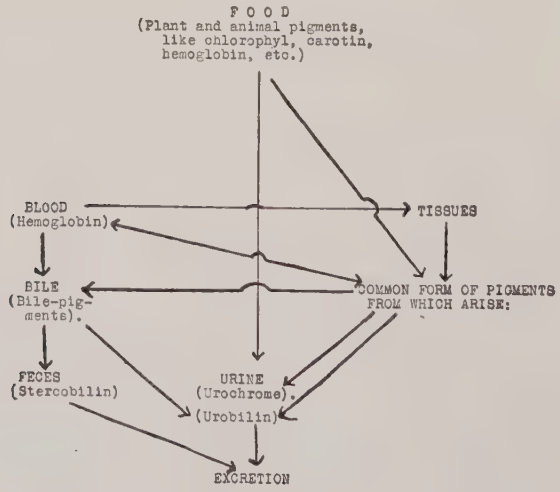
probably produced in the pancreas and finds its way into the urine by excretion from the pancreas into the intestine, there reabsorbed and thence into the urine via kidneys. It is this fact that lends meaning to the clinical observation that when there is a normal amount of amylase in the blood and in the urine, the pancreas is functioning properly. The usual test is to determine the amount of reducing sugar released from a starch or glycogen solution.[1] The methods are based on the original one of Wohlgemuth (1908).

In occlusion of the intestine, an increase in urinary diastase occurs, while in diarrhoea, there is a reduced amount. Urinary diastase varies during the day and night, while blood diastase is quite constant. Day urines are richer in the diastase than night specimens. Moreover, variations occur in diastase content from day to day. For such reasons, the detection of pancreatic lesions by estimations of the diastatic value of the urine is fraught with great difficulties and can be made valid only by prolonged examination.

Urinary Pigments

The relation between the urinary pigments and the body as a whole has been described by Whipple;[2] his ideas are expressed in the following diagram:

Graphic Summary



The term "pigment complex" is used to designate a fundamental chemical configuration for all pigments, whether of blood, urine, feces,

[1] See Dodds, E. C. British Journ. Exper. Pathol., vol. 3, page 133, 1922.

[2] Whipple, G. H. (Rochester School of Medicine, Rochester, N. Y.). See page 487.

or of other sorts.[1] It has not been determined definitely what share the various factors have in the production of urinary pigments; indeed, we do not know the chemical nature of all pigments. The more distinct pigments of the urine will now be discussed:

Normal Pigments. Urochrome.—This is the principal pigment of the urine. It is the main cause of the straw-yellow color of this excretion and of the color of uric acid calculi. It has no readily distinguishable bands in the spectrum.[2] It is derived principally, but not exclusively, from the foods, especially from chlorophyl (but not from carotin, the yellowish pigment of many vegetables). Milk contains a pigment similar to urochrome in that it presents no distinct bands in its spectrum. A substance which does not occur in normal urine, but which appears in certain pathological states has been called "urochromogen." When urine containing this substance is treated with an oxidizing agent like permanganate, the urochrome content is increased. This has led to the belief that a precursor of urochrome exists. Such a precursor has not been isolated. A *test for urochromogen* has been suggested: Five mls. of urine are diluted by adding 15 mls. of water. Add 3 drops of 1 : 1000 permanganate solution; a yellow color develops. Chemically, urochrome has been described as an oxidation product of protids, a so-called "oxyproteïc acid" which contains sulphur, but the knowledge of the acid is incomplete. It is probably not a chemical entity, but a mixture of pigments. The urochromogen is said to appear in intoxications involving high temperatures, as in typhoid fever. It is characteristic of tuberculosis and is frequently used in clinical diagnosis.

It will be observed that urochrome is not derived from bile-pigments.

Urobilin was mentioned in the discussion of the pigments of the bile;[3] it is the "stercobilin" of the feces.

Through the recent researches of Elman and McMaster[4] we are in possession of more definite knowledge of the pigment. It arises only in the intestine. Animals rendered urobilin-free by intubating

[1] For a discussion of these matters, see page 474. The pyrrol ring, four of which occur in hemoglobin, is such a complex.

[2] This statement refers to urine. Drabkin (Drabkin, D. L., Univ. Penna., presented before the Physiological Society of Philadelphia, Feb 21., 1927) however finds evidences of absorption spectra when urochrome is obtained in concentrated form.

[3] Page 476.

[4] Elman, R. and McMaster, P. D. (Rockefeller Institute) Studies in urobilin physiology and pathology. Journ. Exper. Medicine, vol. 42, page 99, 1925.

the common duct remain urobilin-free even after injury to the liver. Urobilinuria never occurs after damage to the liver unless bile is present in the intestine. Bile given by mouth to an animal with ligated common bile-duct, is followed by urobilinuria. "Urobilinuria is an expression of the inability of liver cells to remove from the circulation, the urobilin brought to the portal stream, with the result that pigment passes on to kidney and urine." In the instance of liver injury, urobilinuria occurs with far less degree than does bilirubinuria.

Urobilinogen (mesobilirubinogen) is reduction product of bilirubin by way of mesobilirubin—

$$C_{33}H_{36}N_4O_6 + 2H^2{}_2 \longrightarrow C_{33}H_{40}{}^3N_4O_6 + 2^4H_2 \longrightarrow C_{33}H^5{}_{44}N_4O_6$$

Bilirubin. Reduction. Mesobilirubin. Reduction. Mesobilirubinogen or urobilinogen.

Test for Urobilinogen.—From the practical standpoint no significant physiological or pathological differences exist between urobilinogen and urobilin, and a test which demonstrates the presence of either or of both is, therefore, quite practicable. On the other hand, the fact that urobilinogen does not show characteristic absorption bands in its spectrum, while urobilin does, may be used as a differential character.

Tests for Urobilin and Urobilinogen.—Treat 100 mls. of fresh urine with 90 gs. of solid ammonium sulphate. Let stand half an hour. Filter off the precipitate of urates, urobilinogen, etc., and extract the residue with 300 mls. of ethanol at 55° C. on a water-bath. The ammonium sulphate is precipitated and the supernatant liquid is of a yellow color, due to urochrome. If a few drops of concentrated hydrochloric acid be added to this fluid, the color is intensified, due to the conversion of urobilinogen to urobilin.

Alternate Test of Schlesinger.[1]—To 5 mls. of urine add 1 drop of glacial acetic acid and 2 drops of a tincture of iodin (5 per cent.), which is used to cause the conversion of urobilinogen to urobilin in place of the HCl used above. Now prepare an alcoholic solution of zinc acetate by adding about 1 g. of the acetate to 10 mls. of 95 per cent. ethanol, or, preferably, "absolute, 99.6 per cent.," ethanol. Pour the contents of this tube into the first one containing the urine and iodin, back and forth until the acetate is thoroughly mixed and dissolved. Place yourself near a window, preferably a northern exposure, and with your back to the light, examine the tube for green fluorescence which is positive for urobilin.

[1] Schlesinger, W. (Medical Clinic, University of Vienna, Austria), Deutsch. med. Wochenschr., vol. 29, p. 561, 1903.

Abnormal Pigments

Uro-erythrin,[1] rosaïc acid, uromelanin, or purpurin imparts the deep mahogany color to the highly concentrated urines, especially those of fever patients. The pigment appears in the reddish sediments of such urine, probably owing to its insolubility in water. It is soluble in strong ethanol (99.6 per cent.) and amylol. The chemical structure has not been determined, although it is supposed to be derived from urochrome; others derive it from skatol. Clinically, it is present in the urine in cases of fever, disturbance of liver function, tuberculosis, any chronic condition of the heart and lungs, and bacterial intoxications, except typhoid, although it may accompany the latter. It is especially characteristic of the urine of cases with arthritic involvements (articular rheumatism). Light causes the pigment to fade. Uro-erythrin may be distinguished by its lack of fluorescence; by definite absorption bands which appear between wave-lengths 550 $\mu\mu$ to 480 $\mu\mu$[2] (see chart of spectrum), interrupted at 520 $\mu\mu$ by an indistinct shadow at ordinary dilutions of the urine; by the intensification of the red by concentrated sulphuric acid which probably aids in conversion of a chromogen to uro-erythrin; and by the bluish-green tint given by alkali.

The *test* must be performed in the dark, or in subdued light. Concentrate 100 mls. of the urine on a water-bath; or dissolve some of the sediment tinged with pigment in warm water. To either preparation add ammonium chlorid to saturation.[3] This causes the precipitation of the pigment along with ammonium urate. If time permit, reprecipitation is desirable to remove the urobilin; this is done by filtering off the precipitate, making up the residue to 100 mls., reprecipitating with ammonium chlorid and filtering again. Wash the last residue on the filter with hot water and make up the residue to 50 mls. with distilled water. The solution must now be neutral Then add 50 mls. of chloroform and agitate the solution to remove any hematoporphyrin which may be present. Decant the fluid from the precipitate and acidify it with 1 per cent. acetic acid, 50 mls. Add 50 mls. of chloroform again and extract thoroughly. The chloroform removes the uro-erythrin from the acidulated residue. It may be recovered by evaporating the chloroform on the water-bath. Apply the distinguishing characteristics given above to the residue.

Urorosëinogen (indol-acetic acid), the chromogen of the ordinary pigment, urorosëin, appears in characteristic amounts in pathological

[1] Greek *ouron*, urine, and *erythros*, red.

[2] Figure 124.

[3] Seventy-five grams in hot water to every 100 mls. of fluid.

states (gastritis; pernicious anemia; chlorosis of females at puberty; several unrelated conditions, like typhoid fever and osteomalacia). The pigment, or its chromogen, is soluble in ethanol, but more soluble in amylol; it is insoluble in chloroform, ether, and acetic ethyl ester. It is produced by bacteria in standing urine; for this reason, it is essential that fresh specimens be used, or those preserved with an antiseptic. The pigment is said to appear in normal urine on strictly vegetable diets.

Clinically, its presence is not significant in diagnosis, owing to its appearance in various conditions.

Test.—Treat 5 mls. of fresh urine with 5 mls. concentrated hydrochloric acid. Add 3 drops of 1 per cent. sodium nitrite solution, $NaNO_2$. A rose-red color appears, owing to the oxidation of the chromogen, indol acetic acid, to the pigment uroroseïn.

Bilirubin (hematoidin; perhaps identical with hematoporphyrin), the typical bile-pigment,[1] may appear as a granular sediment varying in color from yellow to reddish or yellowish crystals, rhombic, or needle-like in shape.

The *test* usually employed is extraction with chloroform and application of one of the bile-pigment tests.[2] The crystals, however, are readily recognized. This pigment has little clinical interest, since it does not accompany any one pathological state. The same may be said of a variety of other biliary pigments occurring in the urine.

Hematoporphyrin has been found in many diseases mentioned in the discussion of uroroseïn, and also in sulphonal and other similar poisonings. The dark brownish color of the urine in sulphonal poisoning is, however, only in part due to hematoporphyrin.

The *test* is spectroscopic examination. Preparation of the specimen: To 50 mls. of urine add 10 mls. of 10 per cent. NaOH solution to precipitate the phosphates and pigments, including hematoporphyrin. Decant the supernatant liquid, saving the residue. Treat the residue with 0.5 ml. of concentrated hydrochloric acid and then add 15 mls. of "absolute" ethanol. Filter through dry paper. Transfer 5 mls. of the clear filtrate to a test-tube and examine with the spectroscope. Compare the spectrum with that shown for hematoporphyrin, Fig. 124. The question of the identity of hematoporphyrin with other pigments is an open one (see page 383).

Hemoglobin appears in urine when there is a flow of blood into the urinary tracts and is recognized by the spectroscopic test or the benzidin reaction (page 396). The spectroscopic test always gives

[1] Page 476. [2] Page 478.

hemoglobin, not oxyhemoglobin, in such cases. The hemoglobin becomes changed into other pigments on standing and the presence of hemoglobin indicates a lesion in the lower rather than in the upper tracts.

Homogentisic acid[1] is encountered in alkaptonuria. The chemistry has been discussed. Homogentisic acid appears in acid urines, but to demonstrate its presence an alkaline reaction must be induced either by letting the urine stand, thus permitting the development of ammonia from urea by bacterial action, or by adding 10 per cent. NaOH solution.

Test.—If homogentisic acid is present the urine will give a reduction with copper solutions, like Fehling's; it is necessary, however, to insure that a reducing sugar is not responsible for the reduction; this may be done by fermenting away the glucose in the case of a diabetic urine. Another test is to treat the urine with alkali; a brownish color develops if homogentisic acid is present. This acid is not pathognomonic of any strictly pathological state and has no clinical interest.

The Diazo-reaction of Paul Ehrlich.[2]—Principle: This is probably an oxidation of a chromogen found in pathological urines, but the exact cause of the reaction is unknown. Whatever the nature of the reaction, it is of value clinically, for although it is not pathognomonic of typhoid, it helps to follow the course of the disease, since the intensity of the reaction varies with the severity of the disease. Other conditions in which the diazo-reaction is positive are: Tuberculosis (pulmonary), measles, scarlet fever, diphtheria, and pneumonia. In the arthritides it is generally neutral (compare uroerythrin, page 755).

Reagents: Appendix.

Procedure: Place 100 mls. of the sulphanilic acid solution (I) in a small Erlenmeyer flask and add about 1 ml. of the sodium nitrite solution (II). Introduce into this solution by means of a volumetric cylinder 50 mls. of urine and mix. Pour 5 mls. concentrated ammonium hydroxid carefully down the side of the flask, so that a zone forms. At the line of juncture of the ammonium hydroxid and the solution a red ring appears if the test is positive. Normal urine gives a yellowish-orange ring. Ehrlich adds the following control procedure: Shake the mixture and let stand overnight; a green precipitate appears if positive.

The diazo-reaction may give a falsely positive result following the administration of certain drugs, like opium. It is positive in phenol

[1] Page 268. [2] Ehrlich, Paul (page 273).

(carbolic acid) poisoning. The orange color of normal urine is inhibited by administering tannic acid.

Iminazol derivatives[1] occur in the normal urine. They are derived principally from histidin of endogenous origin, but to some extent from the foods. On a low protid diet 118 mgs. of iminazol substance were found in the urine, while on a high protid diet 172 mgs. were obtained. Pathologically, these substances vary in a characteristic manner in nephritis. They are usually low in such diseases. A method for quantitative determination of iminazoles in the urine is proposed by Koessler and Hanke (page 269, note 2).

Indican.—The chemistry of this substance and the manner of its derivation from intestinal putrefactive products of bacterial origin have been discussed.[2] As an indicator of fecal retention owing to constipation, adhesions, etc., its use is wide-spread. The fact that it appears in certain individuals who are apparently well and have normal stools, makes it improbable that the indican reaction is reliable clinically as its present popularity would lead one to believe. As a rule, a heavy meat diet causes indicanuria, probably for two reasons: (1) The increased amount of tryptophan gives rise to indican, and (2) in persons on high protid diet atony of the intestine and diminished stools are not uncommon. Any static condition of the intestine is conducive to the development of indicanuria, owing to the possibility of bacterial fermentation followed by absorption of the putrefactive products. A common erroneous impression is that in indicanuria the color of the urine is changed from normal. No characteristic color change accompanies indicanuria. However, if the urine is allowed to stand, discoloration develops.

Cotton-plug Test.—Pipette 2 mls. of fresh urine into a test-tube. Stopper the tube with absorbent cotton. Remove the plug, moisten the surface exposed to the contents of the tube with 2 drops of 1 per cent. potassium persulphate ($K_2S_2O_8$) and then with 1 drop of reagent para-dimethyl-benzaldehyde.[3] Now insert the plug again into the mouth of the tube and boil the urine over a microburner for about one minute after the ebullition has begun. The appearance of a pink color indicates the presence of indican.

Obermayer's[4] *Test.*—Principle: The indoxyl-sulphuric acid is oxidized to the condensation product, indigo, which is blue and

[1] Page 272. [2] Page 273. [3] Appendix.

[4] Obermayer, F. (Professor of Internal Medicine, University of Vienna, Austria) Wiener klin. Wochenschr., vol. 3, p. 176, 1890. See page 280.

is soluble in chloroform. Procedure: Place 5 mls. of the urine in a test-tube and add 5 mls. of Obermayer's solution.[1] Stopper the tube and invert it several times. Remove the stopper and add 5 mls. of chloroform. Stopper, shake gently, and then permit the two zones to separate by gravity; the presence of indican is indicated by the blue indigo absorbed by the chloroform layer.

In cases of syphilis in which iodids are administered and are excreted into the urine, the latter is reddish. This color disappears if sodium thiosulphate is added.[2] Urines preserved with thymol turn violet which may obscure the blue of the indigo. Thiosulphate again destroys the color.

Jaffe's Test.[3]—Treat 5 mls. of urine with 5 mls. of concentrated hydrochloric acid. Make, freshly, a dilute solution of calcium hypochlorite $Ca(OCl)_2$ and add 3 drops to the urine solution. Mix. Add 5 mls. of chloroform, stopper, and invert. Indican is oxidized, as before, to indigo, which imparts a blue color to the chloroform. The reaction is frequently only bluish; add more hypochlorite and agitate the solution. Too much hypochlorite will cause the oxidation of the indigo to the yellowish isatinates, chiefly calcium isatinate.

Melanin appears in the urine of persons with melanotic tumors. A substance resembling "melanin" has been described in malaria. In the urine as voided there is a chromogen which, when the urine stands, becomes converted into dark substances. These are probably oxidation products of the aromatic amino-acids, like tyrosin.[4] Ferric chlorid alone without the hydrochloric acid of the indican test causes the melanochromogen to be converted into the dark melanins. This reaction distinguishes melanins from the dark substances which color the urines in cases of carbolic acid poisoning, alkaptonuria, etc.

This completes the survey of nitrogen-bearing substances in the urine. There remain, however, protids and protidtemns, which appear in the urine under pathological conditions.

Protids and Protidtemns.—Protid.—Heat-coagulable protids are absent from normal human urine; the presence of a substance which gives a positive Heller or boiling test[5] is indicative of a pathological condition. In many pathological states protid appears in the urine. It may be similar to if not identical with the serum albumin and serum globulin of the blood, but investigations have failed to show definitely whether urinary protids are derived from the blood or

[1] Appendix.
[2] Making white sodium iodid.
[3] Page 367.
[4] Page 266.
[5] Pages 764 and 763.

whether they arise from the renal tissue.[1] Wells attempted to answer this question by means of immunological reactions, and concluded that as far as this criterion is concerned, there is no evidence that urinary protids are derived from the blood. Chemically, no differentiation has been made between the protids of the blood and of the renal tissue. This much may be said, however: Cells are poor in heat-coagulable protids which would argue for the origin of urinary protids from the blood. Heat-coagulable protids are characteristic of secretory fluids like white of egg and blood protids. Of the urinary protids there are two kinds, namely, albumins and globulins. It is probable that both groups are composed of protids in mechanical mixture rather than a single substance.

Albumin.—In the usual form of nephritis, commonly designated "Bright's disease,"[2] albumin comprises from 25 to 75 per cent. of the total protid in the urine. Since tests are usually insufficiently delicate to detect traces of albumin lower than 0.10 g. per liter of urine, it cannot be said that such traces do not appear in urine which is practically normal, but detectable amounts are always correlated with pathological states, temporary, acute, or chronic. In mild cases of albuminuria from 5 to 10 gs. as dry albumin are excreted. The albuminurias are of various kinds:

1. *Albuminuria of Acute Nephritis.*—The amount of albumin is proportionate to the stage of the disease and the quantitative determination is of clinical importance. The output of albumin is exceptionally high in nephritis of syphilitic origin. In chronic parenchymatous nephritis, or nephrosis, there is a large output of albumin, while in chronic interstitial nephritis the amount of albumin is low and irregular. In renal hypoplasias, little albumin appears. Infections, drugs, agencies causing passive congestion of the kidney, and cardiorenal conditions are attended by the appearance of albumin in the urine. Following athletic events participants frequently have albumin in the urine. Exposure to cold may be the cause of a congestion of the kidneys and consequent albuminuria.

2. *Orthostatic or Postural Albuminuria.*—Albumin appears in the urine of certain individuals when they are caused to stand for a considerable length of time. The ambulance surgeon of the metropolis is frequently called to treat cases of collapse involving albuminuria

[1] Cameron, A. L. (Chicago physician), and Wells, H. G. (Professor of Pathology, University of Chicago), Arch. Int. Med., vol. 15, p. 746, 1915.

[2] Bright, Richard, English physician, died 1858. The name "Bright's disease" is a group name for any form of nephritis in which protid appears in the urine.

when individuals of either sex are required to stand while being measured for clothing in the shops. Since the condition is one of stasis of the blood flow through the kidneys, due to low blood-pressure, exercise frequently affords relief.

3. *Emotional Albuminuria.*—In various nervous diseases (epilepsy, paresis, neurasthenia, etc.) albumin appears in the urine. Experimentally stimulation of the floor of the brain in the region of the fourth ventricle leads to similar conditions.

Still other forms of albuminuria exist which cannot be considered here.

Globulin.—In the blood the ratio $\frac{\text{serum albumin}}{\text{serum globulin}}$ is about $\frac{2.0}{1.0}$. In Bright's disease albumin and globulin appear, but the ratio is seldom that found in the blood. In certain cases, as in hypoplasias of the kidney, there may be more globulin than albumin. In general, an increase in the proportion of globulin to albumin indicates aggravation of the disease. In nephrosis there is a low excretion of globulin and consequently the severity of the disease is indicated by the degree of increase of albumin over globulin.

Protidtemns.—Hydrolysis products of the protids, like albumoses, peptones, and amino-acids, have been observed in the urine under certain conditions. It is questionable that pepton ever occurs, the substance frequently found being albumoses. The term "albumosuria" is applied to the presence of albumose in the urine. This condition arises in the post-critical stage of pneumonia during which the exudate is being absorbed; the autolysis of the pus produces a large amount of albumose which is practically a foreign body and is excreted into the urine. Lesions of the liver are frequently accompanied by the appearance of albumose in the urine. The appearance of amino-acids in urine has already been discussed (page 743).

Protid of Unknown Relationship Discovered by Bence-Jones.[1]—This substance is known as "Bence-Jones Protid." The discoverer considered it a member of the group of primary albumoses, but since it may be hydrolyzed by boiling with acid, and since among the products protalbumose[2] appears, it is not a hetero-albumose as its discoverer believed. It is doubtless a true protid or an early hydrolysis product of protid. Folin[3] believes that it is a protid as low

[1] Bence-Jones, H., English physician, died 1873. Intensive studies of this substance were made by Wilson (Fig. 224). Bayne-Jones, S., and Wilson, D. W., Bull. Johns Hopkins Hospital, vol. 23, pp. 37, 119, 1922; Proc. Soc. Exp. Biol. Med., vol. 18, p. 220, 1921.

[2] Page 343.

[3] See page 762, page 283 of the paper by Folin and Denis.

in the scale as an albumose. "The Bence-Jones albumose is more or less similar to one of the primary peptic digestion products (Meissner's 'metapeptone') and is probably formed by internal autolytic digestion since it appears to be independent of the total protein metabolism." The substance appears in cases of diseases of bone-marrow (myelomas) of the chest and pelvic girdle, in isolated cases of cancer, and probably in lymphatic diseases (leukemia). Clinically, its presence usually indicates multiple myeloma. Few cases have been accurately diagnosed and studied (about 35).

Fig. 224.—D. Wright Wilson, Professor of Biochemistry, University of Pennsylvania, Philadelphia. Investigations of Bence-Jones' substance, acid-base metabolism, etc.

Its *chemical characteristics* are: (1) Its behavior when the urine is heated; at from about 45° to 60° C.[1] turbidity of the urine appears; the opalescence increases to a distinct cloud. At 100° C. this cloud disappears,[2] but returns when the temperature falls to about 60° C.[3] (2) It has been found in 1 case to undergo spontaneous crystallization[4] and this crystalline substance can be recrystallized and purified. Since the proportion of amino-nitrogen to the total nitrogen of the crystalline substance is 4.8 to 100, it is evidently a true protid and not a protidtemn, in which the proportion of amino-nitrogen is much higher.[5] There are probably different kinds of Bence-Jones protids, for Wilson found but one specimen capable of spontaneous crystallization.[6]

[1] Folin and Denis give 40° to 55° C. See Folin, O., and Denis, W., Jour. Biol. Chem., vol. 18, p. 277, 1914.

[2] In a case recently studied by the author the turbidity disappeared at 97° C.

[3] Wilson, D. W. (Fig. 224). Jour. Biol. Chem., vol. 56, p. 203, 1923. See also below, notes 3 and 5.

[4] Gritterink, A., and de Graaff, C. J. W. (Dutch chemists), Zietschr. f. physiologisch. Chem., vol. 34, p. 393, 1902, obtained the Bence-Jones substance in a crystalline condition by special means.

[5] Page 292.

[6] Others have been unable to obtain crystals of Bence-Jones' protid; see Folin and Denis, cited above; also Jour. Physiol., vol. 42, p. 199, 1911.

The *test* for Bence-Jones protid is under Chemical Characteristics.[1]

Crystallizable protids in urine have been described in cases of nephritis.[2] Welker, Thomas and Hektoen found a globular or needle-like crystal in ten nephritic cases which was obtained by fractional precipitation with $(NH_4)_2SO_4$. The crystal seems to belong to the group of serum protids.

Tests for Protids in the Urine.—Delicate tests for protid in the urine are essential in modern clinical diagnosis.

1. *Heat Test.*—Filter 10 mls. of the urine through dry paper; if the urine remains turbid, shake it with a small amount of kaolin and filter again. Or, dilute the urine 1:5 with distilled water. Using a small test-tube ("Wassermann tube"), fill with the urine prepared with either method. Add acetic acid 1:100 until only a slight alkalinity is left. Incline the tube slightly, bring the upper portion into the flame of the microburner, or Bunsen burner turned low, and let the upper portions of the liquid come to a boil. Without agitating the contents of the tube, let it stand a minute or two; a cloud of heat-coagulated protid or of earthy phosphates[3] may appear. In order to differentiate protid from inorganic salts, add 1 drop of 1:10 acetic acid and boil. If the turbidity persists, add another drop and boil again. If still turbid, protid is indicated.[4] Since the coagulum dissolves in higher concentrations of acid, an excess may cause the cloud to disappear and thus give rise to an erroneous conclusion.

2. *The Exton*[5]*-Roch*[6] *Test.*—Place 5 mls. of the filtered urine in a test-tube and add 5 mls. of the reagent;[7] mix by rolling the tube between the palms of the hands. Warm slightly over a low flame. Heating to boiling does not harm the preparation, but adds no value to the test. A match may be used to heat the tube in the absence of another mode of heat. The test is positive if turbidity appears. The

[1] Page 762.

[2] Welker, W. H. (see p. 24), Thomas, W. A. and Hektoen, L. (Director McCormic Inst. for Infec. Diseases, Chicago), Journ. Amer. Med. Assoc., vol. 86, page 1333, 1926. A bibliography of other known cases of crystallizable urinary protids is given. See also note 3, page 762.

[3] Calcium or magnesium phosphates; the boiling evolves CO_2 and permits the precipitation of the salts.

[4] Acid dissolves the phosphates, while coagulated protid does not disperse in the acid medium of this degree of hydrion concentration.

[5] Exton, W. G. (Prudential Life Ins. Co., Biochemical Laboratories, Newark, N. J.), Jour. Amer. Med. Assoc., vol. 80, p. 529, 1923.

[6] Roch, G. (German pharmaceutical chemist), Pharma. Centralhalle, vol. 30, p. 549, 1889. Abstract in Zeitschr. f. Analyt. Chem., vol. 29, p. 241, 1890.

[7] Appendix, p. 926.

physician may use the *test at the bedside*. The solution may be made at any time from the dry ingredients as follows: To 1 teaspoonful of water add as much sulphosalicylic acid as will lie heaping on a United States dime and also as much sodium sulphate, powdered, as will lie heaping on a 5-cent piece.

3. *Heller's*[1] *Test.*—Place about 5 mls. of concentrated colorless nitric acid in a clean test-tube. By means of a pipette[2] overlay the nitric acid with an equal volume of the filtered urine. A zone of coagulated protid appears immediately above the junction of the two liquids if the test is positive.

If the patient has been treated with compound tincture of benzoin to prevent coughing, or for a similar purpose, the gum inhaled, then excreted through the urine, gives a zone similar in appearance to albumin under the conditions of Heller's test. In order to prove the presence of albumin add ethanol to the urine before layering it over the acid; the alcohol dissolves the gum. If the urine is concentrated, a slight formation of urea nitrate may occur. This difficulty is obviated by diluting the urine. Concentrated urine also gives an accentuated development of color due to urobilin, urochrome, and other pigments of urine and of bile in abnormal urine Lastly, certain preservatives, like thymol, behave like gum benzoin in urine treated with nitric acid. Add ethanol or, better, gasolene or other lipid solvent. In general, the zone of albumin can be differentiated at sight from a ring of other substance. Albumin appears as a fluffy zone of ill-defined upper limits, whereas other substances, like those mentioned above, present a more or less definitely defined ring.

4. *Roberts'*[3] *Test.*—To 5 mls. of the reagent in a test-tube add by layering as in (3), one volume of urine. A white zone is positive. Similar precautions to those mentioned for (3) must be taken. The advantage of Roberts' reagent over nitric acid is that colored zones, due to condensations of pigments, do not occur.

5. *Sumner's*[4] *Test.*—To 5 mls. of filtered urine add an equal volume of the reagent.[5] The presence of a precipitate indicates a posi-

[1] Heller, A. L. G., Professor of Pathology, University of Kiel, Germany.

[2] Or, one may partly draw the stopper from the bottle containing the urine, letting the drops flow. Some operators reverse the layering procedure. The so-called "horismascope," designed for ready layering, is obtainable on the market.

[3] Roberts, Sir William, English physician, died 1899. See Appendix for composition of the reagent.

[4] Sumner, J. B., and Hubbard, R. S. (pages 24 and 709), Jour. Biol. Chem., vol. 56, p. 701, 1923. See page 233 and last of this Chapter (Total Base) for other uses of the reagent employed in this method.

[5] Appendix.

tive reaction. The reagent may be diluted if desired. Sensitivity, 1 : 200,000.

Tests for Protidtemns. A heat-incoagulable substance (or substances) occurs in certain degenerative processes such as desquamation of the epithelium of the intestine. Procedure: Heat-coagulate in the presence of acetic acid any true protids that are present. Filter and apply the following tests:

Spiegler's[1] *Test.*—Add 1 drop of concentrated hydrochloric acid and add the acidulated urine to 5 mls. of the reagent in a test-tube, layering as before. A positive test is indicated if a white ring appears. This test is positive for very minute amounts of the protid and for larger amounts of protidtemns (albumoses and peptones). For this reason the separation of coagulable protids must be complete before applying the test.

Quantitative Tests for Albumin in Urine.—1. *Quick's*[2] *Method.*—Principle: Precipitation of the albumin in a known quantity of urine in a specially calibrated tube, similar to an Esbach tube (Fig. 225), and reading the volume of the precipitate in grams per liter of urine. Procedure: Add urine up to the mark indicating 10 mls. capacity and 10 per cent. trichloracetic acid to the mark indicating 15 mls. capacity. Stopper the tube and invert it ten times to insure mixing. Let the tube stand overnight (or longer) and then read the height of the precipitate in grams per liter of urine. The height of the precipitate is directly proportionate to the concentration with a deviation of from 0.05 to 0.5 per cent. The following Table gives the correspondence:

Grams protid per liter.	Volume of precipitate.
0.5	0.5 ml.
1.0	1.1
1.5	1.6
2.0	2.1
2.5	2.6
3.0	3.2
3.5	3.7
4.0	4.2
5.0	5.0

If the precipitate is heavy, the urine should be diluted quantitatively.

[1] Spiegler, E. (Skin specialist and Clinical Assistant, University of Vienna, Austria, died 1908). For composition of the reagent see Appendix. Original description in Ber. d. d. chem. Ges., vol. 25, p. 375, 1892.

[2] Quick, A. J. (Cornell Medical, New York City), Jour. Lab. Clin. Med., vol. 8, p. 615, 1923. The tubes are obtainable from Arthur H. Thomas Co., West Washington Square, Philadelphia, Pa.

2. *Esbach's*[1] *Method.*—Principle: Precipitation of the protid in a calibrated tube by means of Esbach's solution in place of trichloracetic acid. The remainder of the method is the same as that of Quick (1). Procedure: Secure an Esbach's tube and add urine to the U mark and the reagent to the R. Stopper, invert the tube, and let it stand for several hours, preferably overnight. Then read the amount of precipitate. The readings are in grams per liter, or

Fig. 225.—Esbach albuminometer. The tube held in a wooden base is filled to U with urine and to R with reagent. The precipitated albumin is shown as 0.3 per cent. (From McJunkin, Clinical Microscopy and Chemistry.)

tenths of per cent. Quick has shown that Esbach's method gives faithful results only when the concentration of protid is from 2 to 3 gs. per liter of urine; below that amount the readings are too low, and above it, too high. Moreover, temperature exerts an effect; the readings are too when the temperature rises above room temperature (20° C.). The specific gravity of the urine also affects the readings; if the specific gravity is greater than the average normal (1.012), the readings are too low.

[1] Esbach, Franco-Teutonic physician, Paris, who died in 1890. For reagent see Appendix.

3. *Folin and Denis' Method.* This is an adaptation of Kober's nephelometric method[1] for the determination of protids in milk and digestion mixtures. Procedure: To about 75 mls. of water in each of two 100-ml. volumetric flasks add 5 mls. of a 25 per cent. sulphosalicylic acid solution. To one flask add 5 mls. of the standard protid solution.[2] To the second add, in quantities of 0.1 ml. (about 2 drops), the unknown urine containing protid until the two flasks seem to contain about the same amount of protid as far as appearance of turbidity is concerned. Note the number of mls. of urine added. Dilute the contents of the two flasks with distilled water to the marks. Stopper and invert the flasks. Transfer half a colorimeter cup of the standard protid solution to each cup of the instrument and compare the turbidity. The comparisons must be made until one can read within an error of a tenth of a millimeter. Then remove the right-hand cup, drain it free from liquid, and fill half-full with the contents of the second (urine) flask. Set the standard at 20 mms. Adjust the cup containing the unknown until the two fields are alike. Calculation:

$$\frac{\text{Reading of the standard (20)}}{\text{Reading of unknown (n)}} = \frac{\text{Concentration of unknown (x)}}{\text{Concentration of standard (10)}}.$$

$$nx = 200 \text{ and } x = \frac{200}{n} \text{ mgs. of protid in the number of mls. of urine taken.}$$

4. *Kjeldahl Method.*—Place 10 mls. of urine in a beaker and add 2 drops of concentrated hydrochloric acid. Heat slowly to the boiling-point. Filter. Permit the coagulated albumin to remain upon the paper until no more drops of fluid pass to the filtrate. Then throw the coagulated albumin with its paper into a Kjeldahl flask and determine the nitrogen by that method.[3] Multiply the result by 6.25.[4] The result is the number of grams of protid in 10 mls. of urine. The per cent. is obtained by multiplying by 10.

Glucids in the Urine.—Normal urine treated with a delicate reagent that is readily reduced shows the presence of reducing substances. The total reducing substance is about 3 gs. per twenty-four

[1] Kober, P. A., research chemist, G. D. Searle Co., Chicago, Ill. Greek *nephele*, cloud, and *metreo*, to measure. Nephelometry involves rendering the substance turbid and the degree of turbidity depends upon the concentration of the substance. The standard is made in a similar manner and the two preparations are compared in a colorimeter modified in such a manner that all direct light, as in colorimetry, does not pass through the solution, but light sent at right angles to the axis of the cups is caught by the particles of the cloud and sent upward to the eye, Folin, O., and Denis, W., Jour. Biol. Chem., vol. 18, p. 273, 1914.

[2] The method of preparing the standard solution of protid is given in the Appendix.

[3] Page 287. [4] Page 290.

hours. Of this, about 1 g. is due to glucids.[1] Normal urine contains no glucose, but when considerable amounts of it are ingested it seems that a certain level of glucose is reached, whereby the tissues become saturated and the excess sugar appears in the blood and thence in the urine. Benedict found that 20 gs. of glucose taken with a standard breakfast caused an excretion of 0.25 g. of urinary glucid, but that when taken alone no increase in the glucid content of the normal urine occurred. Normally, a person may utilize as much glucose, or equivalent, as he can take into the stomach without nausea. This amounts to from 200 to 300 gs. The test cannot be made with other sugars, like table sugar (sucrose), owing to the difference in rate of absorption of these diglucids from that of glucose. The "paradoxical law of glucose" of Allen[2] states that there is no limit to the amount of sugar which the non-diabetic subject can utilize. The sole limitation is the means of introducing the glucose into the tissues. More than 200 to 300 gs. of glucose cannot be taken by mouth. Woodyatt,[3] however, has found that if glucose is injected into a vein faster than 800 mgs. per kilogram of body weight of the patient per hour, glucose appears in the urine; when this rate is reached the reducing substance in the urine is suddenly increased to 24 times normal. About 50 gs. of glucose given intravenously and without interruption to an average man weighing 70 kilos seems to be the maximal amount the body can utilize per hour. Beyond this limit glycuresis[4] develops. A similar threshold is established for fructose, but galactose and lactose behave differently. There is a degree of tolerance for each individual above which rapidly increasing excretion of galactose occurs as larger amounts of galactose are ingested.[5] For many individuals as much lactose as occurs in a glass of ordinary milk (10 gs.) produces more or less lactosuria or galactosuria.

Folin believes that the glucids which make up the reducing substance of the urine other than that which is due to creatinin, uric acid, etc., are: (1) foreign, non-utilizable glucids introduced into the body in cereals, vegetables, fruits, and (2) decomposition products of glu-

[1] The remainder is made up of uric acid, creatinin, and unknown substances.

[2] Allen, F. M. (Physiatric Institute, Morristown, N. J.). See Glycosuria and Diabetes, Cambridge, Harvard University Press, 1913.

[3] Felsher, H. V., and Woodyatt, R. T. (Sprague Memorial Institute, Chicago), Jour. Biol. Chem., vol. 60, p. 737, 1924. The work was performed on dogs.

[4] Glycuresis, a term due to Benedict to signify an increase, not a new appearance, of urinary sugar. See Jour. Biol. Chem., vol 34, p. 258, 1918.

[5] Berglund, H., and Tsang, G. N., Jour. Biol. Chem., vol. 63, p. xlviii (Proceedings), 1925.

cids due to cooking, canning, baking, and other processes of food preparation. "The sugar of normal urine consists, therefore, of a motley variety of carbohydrate products and carbohydrate derivatives, including di- and polysaccharides."[1] Wolf[2] has given a method by which the glucids of normal urine may be distinguished, the method being the quantitative production of gas (CO_2) from fermentable substances in a Barcroft manometer, by means of an especially active German yeast. In normal urine no fermentable substance was found. More recently, Patterson[3] has corroborated these findings and shown that when normal urine is treated with toluene, a small amount of ordinary baker's yeast and a little phosphoric acid, no fermentation as measured by the manometer is obtained. There is a production, however, of CO_2 if a trace of glucose be added. Patterson made an osazone of the chief urinary sugar. The osazone is quite different from glucosazone. Compared with Fig. 82, page 151, the osazone of normal urine consists of long, linear spicules, M. P. 170–182° C. (M. P. phenylglucosazone 159–160° C.) Greenwald[4] and collaborators find pentose 33 per cent., lactose, and other glucids composing the urinary reducing substances.[5] Diet does not modify the substances excreted on a protid diet, which suggests that the pentoses come from the mononucleotids[6] of the tissues, or of ingested meat and other substances. Concerning the presence of glucose in urine these investigators say: "Glucose may appear in the urine of normal persons after the ingestion of large quantities of glucose, or after the ingestion of a carbohydrate meal immediately following a period of carbohydrate-free diet. Just as with other function, there are probably all gradations in the ability of individuals to assimilate glucose. The existence of a 'glucose threshold' seems established by the work of others," referring to the names mentioned

[1] Folin, O., and Berglund, H., Jour. Biol. Chem., vol. 51, p. 213, 1922.

[2] Page 31. The method is an adaptation of one used by Grafe and Sorgenfrel.

[3] Patterson, J. (Charing Cross Hospital Medical School, London), Biochem. Journ., vol. 20, page 651, 1926.

[4] Greenwald, I., Samet, J., and Gross, J. (Harriman Research Laboratory, Roosevelt Hospital, New York, N. Y.). The nature of sugar in normal urine. I. A Comparison of the Glucose Equivalent of Various Sugars in Different Methods for the Determination of Glucose, Jour. Biol. Chem., vol. 62, p. 397, 1924. Also same number of this journal: II. The Sugar Excretion upon Various Diets and the Influence of Diet upon Glucose Tolerance with Some Remarks on the Nature of the Action of Insulin, p. 401, 1924.

[5] Hynd has shown that Selivanoff's test (page 170) cannot be applied to the urine for detecting fructose owing to the combination of urea with glucose; such a sugar gives a positive Selivanoff test. (Hynd, A. St. Andrews, Biochem. Journ., vol. 20, page 195, 1926.)

[6] Page 339.

above, Folin and Berglund, Benedict and Osterberg, and Felsher and Woodyatt.

While the twenty-four-hour quantity of reducing substance does not vary characteristically with the diet, or with pathological states, the hourly picture has been utilized as a diagnostic agent.[1] Under controlled conditions, if the excretion of urinary reducing substance rises above about 0.10 g. per hour, the metabolism of sugar is pathological.

The effect of insulin on urinary reducing substance is similar to its effect upon the sugar of the blood; the urinary sugar disappears. This fact does not mean that the reducing substance is glucose, because, as Folin suggests,[2] the appearance of glucids in the urine is indicative of a supersaturation of the tissues by such substances. Moreover, since all glucids which are utilizable in the body are converted into glucose, it is readily seen that the effect of insulin, being to immediately reduce glucose, must affect all glucids.

Method for Determining Urinary Glucids. Quantitative Method of Folin and Berglund.[3]—Principle: Interfering substances (creatinin, uric acid, etc.) are removed by Lloyd's reagent[4] by adsorption. Then the di- and polyglucids are treated by acid hydrolysis. The sugars thus produced are estimated quantitatively by the Folin-Wu sugar method.

Procedure: To 5 mls. of the urine add an equal volume of 0.1 normal sulphuric acid and dilute with 10 mls. of distilled water. Add about 1.5 g. of Lloyd's reagent. Agitate the contents for about two minutes. Filter. Pipette 10 mls. of the filtrate into a urea receiving tube graduated at 25 mls.[5] Add 1 ml. of 10 per cent. hydrochloric acid solution. Place the tube in a water-bath at 100° C. Leave it for one hour and a quarter or longer; di- and polyglucids are hydrolyzed to monoglucids. Cool the tube under the cold water-tap. Add carefully in the presence of a piece of litmus-paper, or phenolphthalein, 10 per cent. NaOH solution to neutrality.[6] Add water up to the 25-ml. mark.

[1] Page, I. H. (Eli Lilly Research Laboratories, Indianapolis, Indiana), Jour. Lab. and Clin. Med., vol. 8, p. 631, 1923. [2] Page 769, note 1.

[3] Folin, O., and Berglund, H., Jour. Biol. Chem., vol. 51, p. 209, 1922.

[4] Concentrated "Fuller's earth," a silicious substance found in deposits of fossil unicellular plants called diatoms. Lloyd, J. U., of the firm of Lloyd Brothers, Cincinnati, Ohio.

[5] Figure 226; the tube shown in Fig. 247 is also suitable.

[6] At the neutral point a cloud of material derived from the reagent appears which acts as indicator. If the cloud is used to indicate the neutral point, alkali is added until, after agitating the contents of the tube, the cloud remains.

The determination is made as follows: Place 4 mls. of the solution just mentioned (the dilute filtrate) in a special Folin blood-sugar tube.[1] Transfer to a second and third tube of the same kind exactly 2 mls. each of the weaker (0.20 mg.) and the stronger (0.40 mg.) glucose standard solution, respectively. To each of the three tubes add 2 mls. of the reagent alkaline copper solution.[2] The solution should fill the bulb and some or all of the constricted portion of the Folin tube.[3] Place the tubes in the water-bath at 100° C. for six minutes. Cool the tubes under the tap or in a beaker of cold water. Then add to each tube 2 mls. of the reagent phosphate-molybdate solution.[4] Dissolution of the cuprous oxid requires about two minutes. Dilute each solution to the mark (25 mls.). Stopper and invert each tube in order to mix the contents thoroughly. Compare the colors of the unknown with that of the corresponding standard (weaker or stronger).

Calculation: Place the standard at 20.0 mms. Then:

$$\frac{\text{Reading of standard (20)}}{\text{Reading of unknown (n)}} = \frac{\text{Concentration of unknown (x)}}{\text{Concentration of standard (0.2 mg.)}}.$$

Or:

$$\frac{20}{n} = \frac{x}{0.2}.$$

Solving for x:

$$x = \frac{4}{n} \text{ mgs. for 0.40 mls. urine.}$$

Or:

$$250 \times x \text{ mgs.} = \text{per 100 mls. urine}$$

If the stronger standard is used (0.40 mg.), then $x = 2000 \div n$ mg. per cent. $= \frac{2}{n}$ g. per cent.

Method of Benedict.[5]—Principle: Mercuric nitrate in the presence of sodium hydrogen carbonate is used to precipitate substances like creatin and creatinin which interfere with the reaction by causing reduction. The mercury is removed by means of zinc. The determination of glucids is made upon the filtrate by a modified Lewis-Benedict blood-sugar method.[6]

[1] Figure 252; the tube shown in Fig. 253 can also be used.

[2] Appendix.

[3] Strictly speaking, the fluid should not quite fill the bulb, in order to keep down the effects of CO_2 of the air, but unless special tubes are made having larger bulbs, there will be more fluid than the bulb can accommodate.

[4] Appendix.

[5] Benedict, S. R., and Osterberg, E., Jour. Biol. Chem., vol. 34, p. 195, 1918.

[6] Chapter XVI. The modification consists in increasing the picric acid content of the reagent by making the solution more solvent for that acid. A known quantity of NaOH is used for this purpose.

Procedure: Place 20 mls. of urine in a 500-ml. beaker. Add one volume of the mercuric nitrate solution[1] and mix. Add desiccated sodium bicarbonate in small quantities, while agitating the solution until frothing ceases and blue litmus-paper turns red. Pour the solution upon a dry filter-paper in a funnel and receive the filtrate in a small beaker. Add to the filtrate a pinch of zinc dust and also a drop or two of concentrated hydrochloric acid. Mix and let stand about five minutes. Filter again into a small beaker. Hydrolysis is accomplished according to the procedure given on page 770.

Fig. 226.—The Folin receiving tube for use in urea determinations.

The determination: Transfer 2 mls. of the filtrate to a blood-urea receiving tube similar to that used in the Folin method. Add one volume of water, 1 ml. of 20 per cent. solution of sodium carbonate,[2] and 4 mls. of the reagent picrate.[3] Plug the tube with non-absorbent cotton, place in a water-bath at 100° C., and leave for ten minutes. Cool the tube under the tap, dilute to the mark (25 mls.), and compare in a colorimeter with a standard solution of glucose (1 mg. in 4 mls. of H_2O) which has been treated in exactly the same manner as the unknown (1 ml. Na_2CO_3 sol., 4 mls. picrate; heat ten minutes, and dilute to the mark).

Calculation: With the standard set at 20 mms., $\frac{200}{n}$ gives the milligrams of glucid per 100 mls. of urine.

Detection of Clinically Important Amounts of Glucose in Urine.—We have shown that normal subjects on ordinary diets do not excrete glucose in the urine and there is little doubt that any glucose in the urine is pathognomonic.[4] Aside from "renal glycosuria," which seems to be due to a lesion of the kidney, the statement holds for all forms of glycosuria—alimentary,[5] diabetes mellitus, etc.

The following **qualitative tests** are designed for the practising physician for the detection of glucose in such quantities that they are of importance in the diagnosis of diabetes mellitus, etc.

[1] Appendix.

[2] Or a saturated solution.

[3] Appendix.

[4] Greek *pathos*, suffering, and *gnomon*, an index; that is, indicative of disease.

[5] Alimentary glycosuria is that condition in which glucose appears in the urine after the ingestion of an unusual amount of glucose or glucose-producing foods. It is probable that alimentary glycosuria is an incipient form of diabetes mellitus.

Benedict's Qualitative Test.—To 5 mls. of the Benedict qualitative reagent,[1] in a test-tube, add not over 0.5 ml. of urine. Boil the mixture for not less than two minutes. The change of color from greenish-olive through olive to brown and red indicates a positive reaction, and the concentration of glucose in the urine is roughy indicated by the color; the approach toward the brownish-red indicates larger amounts of glucose.

Folin-McEllroy Method.—Place 5 mls. of reagent in a test-tube and add not over 0.5 ml. of urine. Boil for from one to two minutes. Unlike Benedict's method, the reading for clinical purposes is made on the hot solution. The colors are about the same as those obtained by the Benedict method.

Since neither uric acid not other interfering substances in the urine respond to these qualitative tests, it is unnecessary for practical work to resort to any other method. For this reason the multiplicity of tests for glucose in the urine, that most books dealing with the same subject cover, will not be discussed here.

Ketose Qualitative Test; the Reaction of Selivanoff.[2]—This test demands extreme care in its performance; otherwise aldoses, like glucose, may give a positive reaction.

To 5 mls. of reagent add not more than 0.5 ml. of urine. Boil for about one minute, but not much longer. Place the test-tube in your rack and let it cool to room temperature. The development of color indicates a positive reaction. Urines of subjects on diets high in sucrose give the reaction, especially if the urine is collected within two hours after the meal.

Pentose Qualitative Test of Bial.[3]—Place 5 mls. of the reagent in a test-tube and heat to boiling. Withdraw from the flame and add, at once, 1 ml. of urine. Color develops in the presence of arabinose (derived from foods) or ribose (from nucleoprotid of plant origin, or belonging to the extranuclear mononucleotids[4]). Characteristic absorption bands occur in the spectrum of the solution when cooled.[5]

Lactose Test by Mucic Acid.—Considerable urine is required. Procedure: Transfer about 100 mls. of urine to a low-form beaker and add 200 mls. of colorless concentrated nitric acid. Place the beaker on a boiling water-bath in a hood in order to concentrate the urine. When brown fumes no longer pass off from the beaker, the

[1] Page 160. See also Appendix.

[2] Page 170. For Borchardt's modification see Cole, page 26. For ordinary cases of fructosuria the usual Selivanoff reaction, as described above, is adequate.

[3] Page 169. [4] Page 339. [5] Page 190.

liquid should become clear; this occurs when the volume has been reduced to one-fifth of the original amount. Wash out the contents into a smaller beaker by means of a spray of water. Permit the preparation to stand overnight or longer in a cool place. Examine the contents of the beaker at the next exercise for a mass of small crystals of mucic acid.[1] Place a drop of the deposit in the bottom of the beaker upon a microscope slide, cover with a cover-glass, and examine for characteristic acutely pointed prisms. For certain

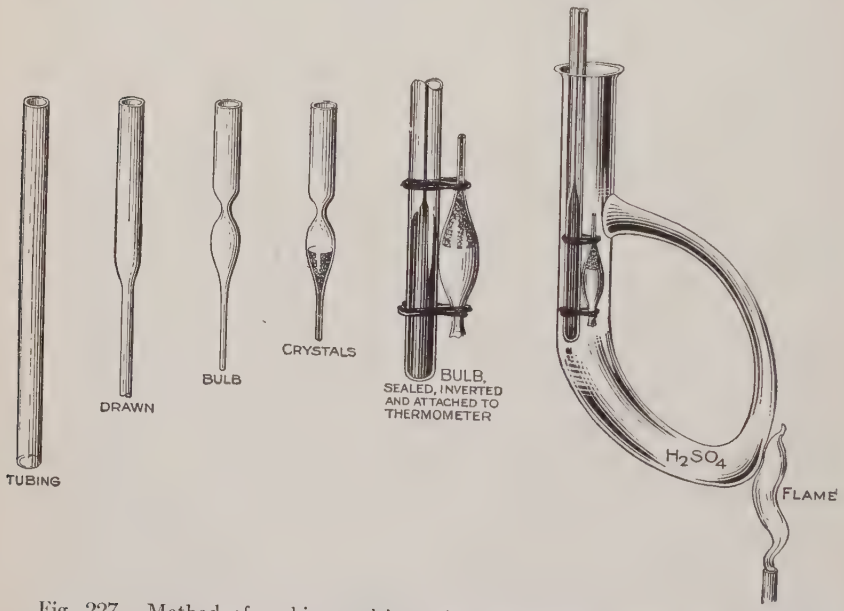


Fig. 227.—Method of making melting-point tubes and of their employment in melting-point determinations. The Thiele tube is used to advantage in this process, as shown. The above method is especially useful in determining the melting-point of osazones.

identification the crystals may be filtered from the liquid, dried in the open air protected from dust, and their melting-point (213° to 215° C.) determined by the method given on page 152.

Osazones are means of identification of urinary sugars. The method for their production is given on page 151. The time of appearance of the crystals is important in diagnosis. Lactosazone appears late, glucosazone quite readily, and maltosazone more readily still. The directions are repeated here:

Phenylhydrazin Test for Glucids in Urine.—Acidulate 10 mls. of urine with 1 ml. glacial acetic acid. Add as much phenylhydrazin

[1] Page 190.

hydrochlorid[1] as will lie upon a United States "nickel" piece and also as much crystalline sodium acetate as will make up thrice that amount by bulk. Dissolve the mixture, using heat if necessary. Filter, in order to remove any undissolved matter. Leave the filtrate in a boiling water-bath for as long as your time permits (half to one hour) and then remove the flame without touching the tube. This insures that the crystals are not broken while forming. After the tube has cooled to about room temperature, carefully pipette[2] some of the residue to a slide and examine without covering the preparation with a cover-glass. Compare the shapes of the crystals with those in the figures. Note the time of appearance of the deposit at the bottom of the tube and apply the statement made on page 774 regarding the time of appearance of these crystals.

Fermentation Test for Glucids.—Pentoses and lactose do not ferment, and it is possible to use this fact as a means of distinguishing these glucids from glucose. See page 175 for general methods. Procedure: Secure a fermentation tube (Fig. 85) and clamp it into your stand. Transfer a test-tubeful of urine to a small mortar, add about one-eighth of a cake of yeast, and with a pestil thoroughly suspend the yeast in the urine. Fill the fermentation tube with the suspension, guarding against any bubbles of air. The bulb is left nearly free from the suspension in order that it may catch the urine displaced by CO_2, formed during fermentation. Place the apparatus in a warm oven, or leave in a warm place until the following period. The accumulation of gas in the upper portions of the tube indicates a positive test for fermenting sugar.

Glucuronates in Urine.—On page 281 we have shown that one of the acids produced by the partial oxidation of glucose is glucuronic acid. Substances are detoxicated by being conjugated with this acid and excreted into the urine as glucuronates. Glucuronic acid can be prepared for class demonstration by either of the following methods:

1. *By Preparation of Saccharic Acid and Subsequent Reduction to Glucuronic Acid.*—Treat a 5 per cent. solution of glucose with enough nitric acid to make a 5 per cent. solution. Leave for one hour. Neutralize with 10 per cent. NaOH solution, taking care to reach the neutral point with litmus-paper. Then add one volume of the following reagent in order to destroy the glucose left unconverted:

[1] If phenylhydrazin base is used, it is a liquid, and 3 mls. are taken. Add an equal bulk of crystals of sodium acetate and a volume of urine of 10 mls. Follow the directions given above for the remainder of this procedure.

[2] Using a large caliber pipette like a 25-ml. pipette or larger, in order that the crystals are not crushed.

Cupric sulphate, three parts by weight, and NaOH (stick), four parts. Let stand a short time and then filter. Save the filtrate. Now reduce the saccharic acid by treating the filtrate with a small amount of sodium amalgam[1] for not longer than five minutes. Then make the solution acid to litmus-paper. The content of saccharic acid is variable, but the solution can be used for the tests.

2. *Method of Plimmer* (see Plimmer, page 220).—Indian-yellow, conjugated glucuronic acid with euxanthone, is obtainable on the market as a synthetic product.[2] Indian-yellow is treated with dilute hydrochloric acid at the boiling-point of water, the solution cooled, the euxanthone filtered off, and the solution neutralized.

Properties of Glucuronic Acid.—The general behavior is similar to that of pentoses, in that it does not ferment with yeast, is dextrorotary in the polariscope, responds to the Bial test for pentoses,[3] and to Fehling's test, but not to Benedict's.

QUANTITATIVE METHODS FOR GLUCOSE IN URINE

I. *Method of Benedict.*[4]—Pipette accurately 25 mls. of Benedict's Quantitative solution into a small porcelain evaporating dish. Add 5 gs. of dry sodium carbonate. Place the dish on an asbestos gauze on a tripod over a bunsen burner.

Now perform a qualitative test (page 160). If the test indicates a considerable content of glucose, dilute the urine 1:10. Otherwise use undiluted urine. Fill the burette with the urine and, as the solution in the dish boils, add, a few mls. at a time, the urine. Continue until the blue color begins to fade, then, proceeding carefully, adding the urine drop by drop, cease the instant a creamy white appearance replaces all the greenish blue. Read the burette. Calculation: The amount of glucose in the number of mls. of urine added to the evaporating dish from the burette is 0.050 g.[5] In order to calculate the 24 hour quantity find the amount of glucose in 1.0 ml. of the urine and multiply by the 24 hour amount. Example: The burette reading is 17.6 mls., urine vol. 1250.

$$\frac{0.050}{17.6} \times 1250 = 3.56 \text{ gs.}$$

[1] A larger amount or a prolonged exposure of the filtrate to this reducing action converts most of the saccharic acid to gluconic acid.

[2] Sulphanilic acid is diazotized and diphenylamin is added; the compound formed is treated with nitric acid. When herbivores, like the rabbit, are fed the leaves of the Indian mango-tree euxanthic acid (Indian-yellow) is formed by detoxicating euxanthone with glucuronic acid. The compound is hydrolyzed as described above.

[3] Page 169.

[4] See page 160.

[5] If the sugar is lactose, the amount necessary to reduce 25 mls. of Benedict's solution is 67 mgs.

II. *Method of Folin and McEllroy.* –Principle: The method is a modification of the Fehling method in which the cupric hydroxid is held out of solution by phosphoric acid in place of Rochelle salt (Fehling), sodium citrate (Benedict), etc. (page 159). Reagents. See Appendix. Procedure: Into a 200 × 20 mm. Pyrex test-tube pipette, accurately, 5 mls. of the Folin-McEllroy cupric-sulphuric solution and 1 ml. of the 20 per cent. sodium carbonate solution. Add 5 gs. ± 0.5 g. of the alkaline phosphate mixture. Heat the tube gently over a micro-burner in order to dissolve the mixture; this requires about 60 secs. at 60° C. From your burette[1] filled with urine, add from 5 to 30 drops to the test-tube. Warm the test-tube gently if it has been cooled and continue heating to distinct boiling. With watch in hand, note the second-hand as soon as boiling has begun and maintain such heating that the solution just boils. A quartz pebble may be added to prevent bumping. Note at the end of 15 secs. whether a precipitate has occurred causing the contents of the tube to become turbid; if not, about one-half of the amount of urine necessary to reduce the copper has been added. Continue adding a few drops of urine at a time until the turbidity has appeared; about 5 secs. are required to cause precipitation after boiling has begun if sufficient sugar is present. The total time of boiling should not exceed 7 mins. and not less than 4 mins. After this preliminary determination, repeat in order to obtain more exact figures. The following table gives approximate boiling times for different amounts of glucose:

Glucose.	*Time.*
50 mgs.	25 secs.
40 "	40 "
35 "	55 "
30 "	80 "
27.5 "	90 "
25.5 "	Three minutes.

Calculation: Five mls. Cu = 25 mgs. glucid $\frac{2.5}{\text{mls. urine used}^2}$ = gs. per 100 mls. urine.

[1] A special sugar burette delivering 25–30 drops per ml., capable of being read to 0.01 ml., and holding 5 mls. is manufactured after the specifications of Folin and McEllroy (Journ. Biol. Chem., vol. 33, p. 513, 1918 and vol. 38, p. 287, 1919).

[2] A method somewhat similar to the preceding, depending upon the time of appearance of the white precipitated cupric thiocyanate and, if necessary, back titration by hydroxylamin, has been described by Cole. The method is rapid and accurate. Details are given in Cole, S. W., Practical Physiological Chemistry, 7th ed., Cambridge (England), W. Heffer and Sons Ltd., 1926, page 180.

III. *Method of Sumner.*—Principle: 3-5-Di-nitro-salicylic acid (page 233) is reduced by glucose to 3-nitro-5-amino-salicylic acid, reddish in color. This color is compared to that of a ferric-ammonium sulphate standard solution. Reagents: See Appendix, page 925. Procedure: Secure a Folin blood-sugar tube and pipette one ml. of urine into it. Add 3 mls. of the salicylic acid reagent. Mix. Leave 5 minutes in a water bath (boiling). Cool, dilute to the mark (25 mls.), mix and compare against the standard iron-alum solution, which is equivalent to 1 mg. sugar.

Lipids, Lipidtemns, and Their Oxidation Products in Urine.— Fat occurs in the urine under unusual, pathological conditions such as lymph varicosity in the kidney, bladder, or elsewhere in the genito-urinary tract; the presence of the parasite Filiaria, an Oriental nematode worm; and postural strains. These conditions are probably induced by lymph stasis[1] resulting from inefficient lymph movement. The average amount of fat found in the urine under such circumstances ranges from 0.5 to 2.2 gs. per 100 mls. of urine.[2]

Method of Qualitative Detection of Fat in Urine.—Pipette 5 mls. of the urine into a test-tube and add 1 volume of carbon tetrachlorid, stopper the tube, and invert it several times. Leave five minutes and then repeat the inversion. Let the two layers separate. Decant the upper layer of the tetrachlorid from the lower by means of a pipette. Pour the tetrachlorid into a small beaker and leave it in a water-bath at 100° C., or on an electric hot-plate at low heat, over an asbestos mat until all of the tetrachlorid has evaporated. If a residue remain, perform Dunstan's[3] test.

Principle: When polyhydric alcohols, like glycerol,[4] are added to an aqueous solution of sodium pyroborate, $NaBO_2.B_2O_3$, the following reaction takes place:

$$\underset{\text{Glycerol.}}{2C_3H_5(OH)_3} + \underset{\text{Sodium pyroborate.}}{2NaBO_2.B_2O_3} \rightleftarrows \underset{\text{Glycerol "borin."}}{2C_3H_5BO_3} + \underset{\text{Sodium metaborate.}}{2NaBO_2} + 3H_2O.$$

The reaction resembles that of the borax-bead, as when copper and borax are heated in a flame, giving $2NaBO_2.Cu(BO_2)_2$. In aqueous

[1] Latin *sto*, I stand.

[2] Sometimes "fat" has been found in the urine after catheterization; the "fat" is petrolatum used in lubricating the catheter.

[3] Dunstan, W. R. (Professor of Chemistry, Pharmaceutical Soc. of England), Note on the Reaction of Glycerin and Other Polyhydric Alcohols, Pharm. Jour., Series 3, vol. 13, p. 257, 1882. The reactions are given in full in Jour. Chem. Soc., vol. 46, p. 278, 1884.

[4] Other substances responding to this test are mannitol, erythritol, pyrogallol, and guaiacol, as well as glucose, levulose, and lactose.

solution, the glycerinborin is hydrolyzed to boric acid: $C_3H_5BO_3 + 3H_2O \longrightarrow C_3H_5(OH)_3 + H_3BO_3$, giving an acid reaction. Procedure: Add sufficient phenolphthalein (indicator) to 5 mls. of 0.5 per cent. borax to produce an evident pink color. Then add this solution to the residue from the tetrachlorid, slowly, drop by drop, until the pink color is lost, after which heat to boiling. The color reappears if glycerol is present.

In place of the test just given, the acroleïn test may be applied.[1]

Quantitative Determination of Lipids in the Urine.—Pipette 10 mls. of the urine into an alundum extraction thimble[2] and place at once in the middle compartment of a Soxhlet[3] extraction apparatus (Fig. 143). Fill the flask attached to the lower portion of the apparatus two-thirds full of ether and, after fixing it in place, turn on the electric current. The ether boils and the vapors condense in the middle compartment. The ether accumulates until it fills the compartment to the smaller siphon tube; it then siphons to the flask below, carrying with it the dissolved fat. After siphoning five times, turn off the current, slip an asbestos mat under the flask, let the ether accumulate in the middle compartment, and when it has just about reached the outlet of the siphon tube, remove the flask and substitute a second flask, or beaker. Place the original flask in a hot-water bath and leave until all the ether has been removed. The residue contains water and any fat which may be present. Place the flask on an electric hot plate and leave until the water and ether have entirely vaporized. Cool the flask, wipe the exterior free from any adhering water, and weigh the flask to tenths of a gram. Then add 5 mls. of ether, heat gently in a water-bath, and pour off the liquid. Repeat three times. Cool the flask and weigh again. The difference in the two weights gives the fat content in 10 mls. of urine.

Products of Lipidtemns Occur in the Urine under Pathological Conditions.—It is true that many urines, when examined very carefully, are found to contain "acidosis" substances, like aceton, but it is probable that in each case there is ketosis,[4] due to diet, exertion, or

[1] Page 199.

[2] Or use a piece of filter-paper thrown into a scroll, into which the urine is pipetted until the paper is saturated; then the paper is dried and more urine is added to completion of the 10 mls. The dried paper contains the fat. In drying, the temperature must not exceed 40° C.

[3] Dr. Henry Leffman, Pathological Chemist, Emeritus, Jefferson Hospital, has called the author's attention to the fact that this method should be called the Chwambathy method, after Soxhlet's pupil by that name. In the original paper Soxhlet distinctly says that the invention was made by his pupil.

[4] Page 531.

to some other factor. Hubbard[1] has shown the variations under controlled conditions. Normally the fats are burned to CO_2 and H_2O. In cases of anoxemia and of diabetes oxidation does not go on to completion, but stops at the 4-ca bon stage. Beta-hydroxy-butyric acid, aceto-acetic acid, and aceton are formed. These acids are then mostly excreted into the urine, although some may pass off in the breath.

Qualitative Tests for Acidosis Substances in the Urine.—Aceto-acetic acid soon disappears from urine on standing. For this reason tests to detect its presence should be undertaken as soon as possible after the urine is voided, and if the tests for other substances are to be made, the test for aceto-acetic acid should be made first.

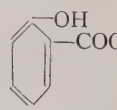
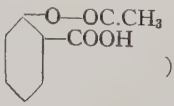
Aceto-acetic Acid.—The most faithful test is that of Gerhardt.[2]

Principle: Aceto-acetic acid[3] in its enol form reduces ferric chlorid, Fe_2Cl_6, to ferrous chlorid, $FeCl_2$; ferrous chlorid is oxidized to the greenish or brownish ferrous hydroxid, $Fe(OH)_2$. The mixture of these various salts has a red color which is that obtained in the test. A preliminary treatment of the urine with ferric chlorid causes the urinary phosphates to be precipitated as ferric phosphate, and this compound is removed by filtration. Procedure: To 5 mls. of the urine add, drop by drop, 5 per cent. ferric chlorid solution until no more precipitate is obtained. Filter. To the filtrate add a few drops more of ferric chlorid solution. A positive test is given by the appearance of a maroon color in the liquid.[4] The depth of color does not ordinarily indicate the quantity of aceto-acetic acid with any faithfulness.

[1] Hubbard, R. S., and Wright, F. R., Variations in the Rate of Excretion of the Aceton Bodies During the Day, Jour. Biol. Chem., vol. 61, p. 377, 1924. The figure on page 381 of this article is referred to. See page 23.

[2] Gerhardt, C. F., French chemist, died 1856. See Bigwood, E. J. (Johns Hopkins University, Baltimore), and Ladd, W. S. (Columbia University, New York, N. Y.), Jour. Biol. Chem., vol. 58, p. 347, 1923.

[3] Also called di-acetic and acetyl-acetic acid, $CH_3.C:O.CH_2.COOH$ (keto form), and $CH_3.COH.CH_2.COOH$ (enol form).

[4] Substances which carry an hydroxy group, similar to the enol of aceto-acetic acid, give the same color with ferric chlorid. Salicylic acid,  , is an example. Aspirin (acetylsalicylic acid, ), frequently administered as an analgesic, is slowly converted into salicylic acid in the body, and this acid is excreted into the urine. For this reason it is necessary to remove the salicylates

Arnold-Lipliavsky[1] *Test for Aceto-acetic Acid.*—Dissolve a crystal (the size of a buck-shot) of para-aminoacetophenon[2] in five drops of 5 per cent. HCl solution in a test-tube. Add 10 mls. urine, a piece of sodium nitrite, $NaNO_2$ and three drops of concentrated (desk reagent) ammonium hydroxid. Agitate vigorously. Then, transfer about 2 mls. of the solution to another tube (200 × 20 mm.), add 15–20 mls. of concentrated HCl, 3 mls. chloroform and 3 drops of 10 per cent. ferric chlorid solution. Mix carefully and gently and let stand two minutes. In the presence of aceto-acetic acid, the chloroform layer becomes violet or bluish. A negative test results in simply a yellowish or weak rose-color. Aceton gives no changes of color in a ferric chlorid solution. This permits us to distinguish between these two acids, especially when the test is made in conjunction with the following one, in which sodium nitroprussid is used; this substance reacts with both aceto-acetic acid and aceton. If the above test is negative while the following one is positive, aceton is indicated. If the following test is strongly positive, coming immediately, aceto-acetic acid is indicated, and the test of Gerhardt should be performed.

Bigwood-Ladd Test for Aceton.[3]—Using the special solution,[1] add 20 drops to 10 mls. of urine in a test-tube. Stopper the tube, invert it several times, and then pipette down the side of the tube 5 mls. of concentrated ammonium hydroxid solution. If the test is positive, a purplish zone appears at the junction of the two liquids. The preparation should stand for two minutes before being read. The following reports are suggested:

Trace	Yellowish ring, no purple.
+	Narrow zone of purple.
++	Wide ring, not intense in color.
+++	Wide ring, maximum.
++++	The ring is a heavy purple mass.

by shaking the urine previous to the test with chloroform; then acidify the solution with concentrated H_2SO_4 and add ether. After shaking the preparation and permitting the liquid to settle, the supernatant ether layer is pipetted into a test-tube, ferric chlorid is added, drop by drop, to the ether, and if the test is positive for aceto-acetic acid, the maroon color appears.

[1] Arnold, V. (Krankenhaus, Lemberg, Germany) Wiener klin. Wochenschr., vol. 12, page 541, 1899. Lipliavsky, S. (Berlin), Deutsch med. Wochenschr., vol. 27, page 151, 1901.

[2] $H_2N.C_6H_4.CO.CH_3$, an aromatic keton. Obtainable from Eastman Kodak Co., as No. 631, M. P. 104–5° C.

[3] For reagents see Appendix.

Quantitative Method for Total Aceton Substances (Van Slyke). – Principle: After removing interfering substances (glucose, etc.)[1] by cupric sulphate and calcium salt, the substances are oxidized to aceton by means of potassium dichromate. The aceton is weighed as mercuro-sulphate-chromate compound.

Procedure: Precipitation of the glucose. Transfer 25 mls. of urine to a 250-ml. volumetric flask or cylinder. Dilute with 100 mls. distilled water and 50 mls. of the special cupric sulphate solution.[2] Mix by stoppering and inverting the flask or cylinder. Add 50 mls. of the special $Ca(OH)_2$ suspension. Mix. If still acid to litmus, add an additional amount of the hydroxid and distilled water to make 250 mls. of solution. Let the preparation stand for thirty minutes. Filter the precipitated glucose from the solution by passing it through a folded filter-paper (Fig. 218), or a physiological funnel,[3] without folding the paper into flutings.

The determination: Pipette 25 mls. of the filtrate obtained from the above treatment into a 400-ml. Erlenmeyer flask, or a small "balloon" flask (Fig. 262). Dilute with 100 mls. of distilled water. Add 10 mls. of half-concentrated sulphuric acid and 35 mls. of 10 per cent. solution of mercuric sulphate, $HgSO_4.2H_2O$. Insert a stopper bearing a Hopkins reflux condenser (Fig. 112) into the mouth of the flask. Place the flask on a wire gauze over a Bunsen burner and heat the solution to boiling. Continue boiling for an hour and a half, or longer. Then let the flask cool at first in the air and then under the tap. Filter the solution by suction through an alundum filter-crucible, saving the residue. Not over 200 mls. of cold distilled water may be used to wash the residue under suction. Let the air current pass through the crucible for as long as possible, then remove the crucible and place it in the thermostat at 110° C. for one hour, or until the following period. Then let it cool in the open air. Weigh the crucible containing the precipitate. Then remove the precipitate and wash thoroughly with water. Place the crucible on a pipe-stem triangle over a Bunsen burner and let it remain for half an hour. Then rinse the crucible, let it dry in the air, and weigh again. The difference gives the weight of the mercury compound.

[1] It is not necessary that the urine be diabetic to contain these interfering substances. Glucids, creatinin, etc., interfere.

[2] Appendix.

[3] A funnel with corrugated sides to prevent paper from adhering closely to the glass and inhibiting filtration. Sometimes the term is applied to a very small ordinary funnel (page 496).

Calculation: Estimate the total aceton substances as aceton: Twenty milligrams of the mercury are equivalent to 1 mg. of aceton. In order to obtain the total aceton substances in 100 mls. of urine divide the weight of the mercury compound (found as described above) by 0.02, and multiply the result by 40.[1]

Alternate Method of Determination. -Titration of the mercury by potassium iodid: After the crucible has been taken from the oven and permitted to cool in cold water, transfer the contents to a small beaker, and add 15 mls. of normal hydrochloric acid solution. Dissolve the mixture by heating. Cool and add about 6 mls. of 3 M (thrice molecular) sodium acetate, which acts as a buffer and reduces acidity. Place the beaker beneath a burette containing 0.2 M potassium iodid solution and titrate until the end-point has been reached, which is indicated by the solution of red mercuric iodid after it has been formed. It is best to add an excess of the iodid and to titrate back with a known solution of mercuric chlorid (0.05 M $HgCl_2$ solution).[2]

Calculation: One ml. of the 0.20 M potassium iodid solution is equivalent to 13 mgs. of the mercury precipitate.

Determination of Aceton by the Turbidimetric Method of Folin.— Principle: The aceton is aspirated into a solution of bisulphite. The compound thus formed is suspended in an alkaline mercuric cyanid-$AgNO_3$ solution, the turbidity then being compared with that developed by a standard solution of aceton. Procedure: To about 1 ml. of 10 per cent. sulphuric acid solution in a 200 × 20 mm. test-tube, add 2 mls. of urine.[3] Place in a similar tube 10 mls. of freshly prepared 2 per cent. sodium bisulphite solution ($NaHSO_3$).[4] Connect the two tubes as shown in the figure (Fig. 241). Place the tube containing the urine in a beaker of water at about 40° C. Now start

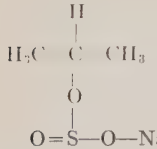
[1] That is, 25 mls. of urine were taken originally; this makes 4 × 25 = 100. Of the 250 mls. in the filtrate from the precipitation process, 250 mls. were taken. This makes the dilution 25 to 250 or 1:10. Then 4 × 10 = 40.

[2] The mercuric chlorid solution should be standardized against the potassium iodid used. Pipette exactly 25 mls. of the 0.05 M $HgCl_2$ into a small beaker, *taking care that none of the fluid enters the mouth!* Dilute with about 100 mls. of distilled water. Pass H_2S gas through the solution until the maximum black mercuric sulphide, HgS, has been obtained. Filter this by suction from the filtrate, and weigh as directed above. There should be 0.2908 g. of mercuric sulphide in the weighed amount. Then the potassium iodid solution is titrated against the standard mercuric chlorid solution, the exact concentration of which has been just determined by the sulphide method.

[3] The amount should be such as to give about 0.5 mg. aceton.

[4] This substance may be obtained from dealers in photographic supplies. The commercial solution contains 50 per cent. bisulphite. It must be kept stoppered.

the air current, which carries the aceton over into the bisulphite solution, and let it run for ten minutes. Aceton-sodium-bisulphite,



is formed. Pour the solution quantitatively into a 100-ml. volumetric flask, washing with a small amount of distilled water, enough to make a volume of about 50 mls. of fluid. The standard is made as follows: To each of the two similar volumetric flasks add 10 mls. of the standard aceton solution containing 0.5 ml. of aceton; add also 10 mls. of 2 per cent. sodium bisulphite solution, and dilute with distilled water to make a volume of about 50 mls. Now add to each flask 15 mls. of the alkaline $Hg(CN)_2.AgNO_3$ solution[1] which must be clear. Dilute at once with distilled water to the mark. Mix and let stand for about fifteen minutes. Place the colorimeter to face a window and pull the curtain down within about a decimeter of the sash, so that the light emerges from a horizontal slit. Make sure[2] that each field of the colorimeter is illuminated equally with the other. Place about 5 mls. of the suspension from one standard preparation in the left cup and a similar quantity of the unknown in the right cup. Set the standard at 20 mms. Calculation:

$$\frac{\text{Reading of standard (20)}}{\text{Reading of unknown (n)}} = \frac{\text{Concentration of unknown (x)}}{\text{Concentration of standard (0.5)}}.$$

Or

$$\frac{20}{n} = \frac{x}{0.5};\ x = \frac{10}{n} \text{ for 2 mls. and } x = \frac{500}{n} \text{ mgs. per cent.}$$

Determination of Beta-hydroxy-butyric Acid.—This may be determined by ascertaining the difference between the total aceton substances and the quantity of aceto-acetic acid determined by the foregoing method. For direct determination of beta-hydroxy-butyric acid, proceed as follows:

Folin-Shaffer-Marriott Method for β-hydroxy-butyric Acid.—P. A. Shaffer[3] estimated the amount of β-hydroxy-butyric acid by causing it to become oxidized to aceton. Marriott[4] adapted this method to

[1] Appendix.

[2] This is best done by filling each cup of the colorimeter with the standard solution from one of the standard flasks and placing the left cup at 20 mms.; read the right cup against it. If the readings do not agree, adjust the colorimeter until they do.

[3] Fig. 163.

[4] Marriott, W. McK., Professor of Pediatrics, Washington University, St. Louis, Mo.

the determination of β-hydroxy-butyric acid in blood. Folin combined these two methods and incorporated the more recent work of Shaffer, which showed that when small amounts of the acid are oxidized by chromic acid, theoretical yields of aceton are obtained. Procedure: Diluting the specimen, pipette 5 mls. of urine into a 100-ml. volumetric flask and fill to the mark with distilled water. This gives, on the average, about 2 mgs. of β-hydroxy-butyric acid per ml.

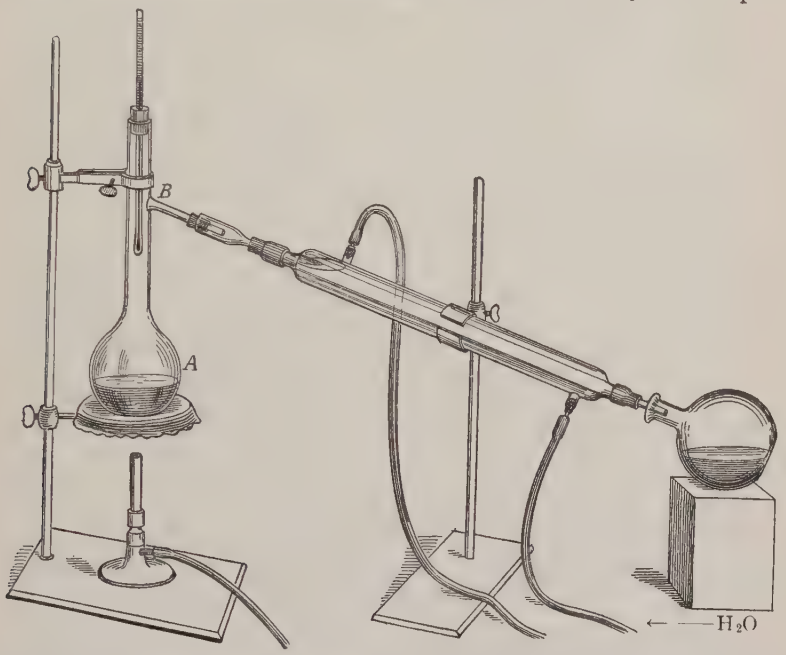


Fig. 228.—Apparatus for distillation. Water is passed through the apparatus by means of the lower (right-hand) tubing and out into the sink through the upper tubing. (From Holland, Medical Chemistry and Toxicology.)

of solution. The determination: Pipette 2 mls. of the urine thus diluted into a large Kjeldahl flask. Add 200 mls. of water and 5 mls. of 10 per cent. sulphuric acid solution. Boil over a free flame for about ten minutes.[1] Measure (cylinder) 25 mls. of the special chromic acid solution.[2] Arrange the flask for distillation by connecting it to the Reitmeyer adapter bulb of a Kjeldahl distilling apparatus.[3] Place a pint milk bottle below the apparatus, as in the Kjeldahl method,[4] but in place of the acid usually used in the Kjeldahl

[1] The purpose of this procedure is to remove acetone or aceto-acetic acid which is nearly always present in urine containing β-hydroxy-butyric acid.

[2] Appendix.

[3] Figure 228; the Reitmeyer bulb is shown in Figs. 110 and 111.

[4] Page 287.

method add about 100 mls. of distilled water to the bottle. Make sure that the tube leading from the end of the condenser to the bottle dips beneath the water in it. Light the burner and keep it turned low for about half an hour; then increase the flame At no time is it necessary to cause the contents of the flask to boil actively, owing to the high volatility of aceton (56.1° C.). At the end of an hour disconnect the flask, pour out the contents, and rinse with distilled water. Transfer the contents of the milk bottle to the Kjeldahl flask and add about 2 gs. of sodium peroxid. Reconnect the Kjeldahl flask with the apparatus and distill about 80 mls. again into the milk bottle, which contains about 10 mls. of distilled water. The second distillation takes about fifteen minutes. Disconnect the milk bottle, turn off the flame, and then add the contents of the bottle to a 100-ml. volumetric flask. Dilute to the mark, stopper, invert, and mix. Pour the contents of the flask into a beaker. Rinse the flask twice with distilled water and then pipette 50 mls. of the contents of the beaker into the flask. Add to the flask 15 mls. of the Scott-Wilson solution,[1] dilute to the mark with distilled water, stopper, invert, and mix. To a second 100-ml. volumetric flask add 10 mls. of the standard aceton solution used in the previous determination[2] and also 15 mls. of the Scott-Wilson reagent. Dilute the contents of this flask to the mark with distilled water. Let the two flasks stand for fifteen minutes. Then compare the turbidities in a colorimeter as directed before.[3] Calculation: The content of aceton per mls. of unknown urine specimen is obtained exactly as in the preceding determination.[4] In the present case, however, the urine is diluted 1 to 20 and it is necessary to multiply the result by 20 in order to obtain the amount of aceton in 1 ml. of urine; as only one-half of the distillate (50 mls.) was taken for turbidimetry. This figure multiplied by 1.85[5] gives the β-hydroxybutyric acid corresponding to the number of milligrams of aceton found.

Iorganic Constitutents of the Urine

From the standpoint of quantity the most important constituent of the urine, aside from urea (30 gs. per day), is *sodium chlorid* (15

[1] Appendix.
[2] Page 784.
[3] Page 784.
[4] Page 783.
[5] This is the amount of β-hydroxy-butyric acid that corresponds to 1 mg. of acetone. The ratio of the molecular weights of β-hydroxy-butyric acid and acetone is $\frac{104.08}{56.06} = 1.85$.

gs. per day). Other mineral substances are excreted in the urine, but none approaches sodium chlorid in quantity. The following Table gives the concentrations and probable function of the inorganic substances of the urine:

Substance.	*Quantity per twenty-four hours.*	*Probable function.*
Sodium chlorid, NaCl	15 gs.[1] 9.4 gs.[2] 8.5 gs.[3] 8.42 gs.[4]	Solvent; proper osmotic relations; aids in maintaining reaction.
Phosphoric acid, H_3PO_4	1.6 gs. (Folin) 3.5 (Abderhalden)	As NaH_2PO_4 reduces the acidity of the body; when retained, owing to impairment of the kidney in nephritis, the alkali reserve of the blood is depleted, as in ketosis. A small amount of organic phosphorus is excreted by way of the urine. This represents compounds like nucleoprotids, phosphoprotids, and phospholipids.
Sulphur (as H_2SO_4)	2.1 gs. (Folin) 3.2 (Abderhalden)	Represents metabolism of the protids and aids in the regulation of the body neutrality by excreting the acid radicle $SO_4^{=}$.

Total sulphur is divisible into three kinds of sulphur (page 590):

1. Inorganic, due to the oxidation of sulphur in the liver; this is of two kinds:
   (*a*) Derived from the cystin and other organic sulphur of the foods; the sulphur becomes oxidized in the liver.
   (*b*) Derived from the sulphates of the food.
   Amount of inorganic sulphate, 2.7 gs. per twenty-four hours.
2. Ethereal sulphate, due to inorganic sulphur conjugated with aromatic substances from foods, intestinal putrefaction, etc.
   Amount of ethereal sulphate, 0.25 g.
3. Sulphur which has not been oxidized in the liver; this consists of that cystin and other sulphur-bearing compounds derived partly from foods, which have escaped destruction in the liver; and similar compounds derived partly from the tissues.
   Amount of "neutral sulphur," 0.16 g.

[1] On a salt intake of about 18 gs. per twenty-four hours, about 15 gs. NaCl are excreted. This is normal for many persons. Concerning reduced amounts of NaCl in the diet see page 572.

[2] Abderhalden, E. (Tr. by W. T. Hall), Text-book of Physiological Chemistry, New York, John Wiley & Sons, 1908, p. 590.

[3] Folin, O., Proportionated from tables in Folin, p. 121 of his book (see page 26).

[4] Mosenthal, H. O., Renal Function as Measured by the Elimination of Fluids, Salt and Nitrogen, and the Specific Gravity of the Urine. II. The Effect of High, Low, and Normal Diets, Arch. Int. Med., vol. 22, p. 770, 1918. The intake was 8.5 gs. per twenty-four hours.

Substance.	*Quantity per twenty-four hours.*	*Probable function.*
Sodium, Na	4 gs.	Excreted with cations like Cl^-. Concerned with the factors mentioned above under NaCl.
Potassium, K	2 gs. as K_2O	Represents the metabolism of the tissues as contrasted with the intercellular material. Becomes excreted in proportion to sodium as 1:3.5. Potassium is necessary in the maintenance of proper osmotic relations.
Calcium, Ca	7 gs.	Excreted as calcium phosphate, chiefly. The exact rôle of Ca in excretion and in metabolism as a whole is not known. The amount excreted varies with the excretion of other inorganic substances, like phosphates and chlorids.
Magnesium, Mg.	0.30 g.	Magnesium resembles calcium in its occurrence and association with other excreted substances.
Iron, Fe	0.01 g.	The minute amount of iron excreted in the urine is insignificant at the present time for physiological or clinical purposes.

Gases.—Oxygen (always in physical mixture) occurring in human urine to the extent of 0.33 volume per cent., equivalent to a tension of 72 mm. Hg.[1] The CO_2 content is about 6.5 vol. per cent.[2]

Besides the substances mentioned above, other inorganic elements like iodin have been detected. To date, no definite relation between their presence and physiological or pathological variations has been discovered.

Methods for the Determination of Inorganic Substances in the Urine

The following methods have been selected principally owing to their utility in clinical diagnosis. Rapidity of procedure, small amounts of fluid required, and similar factors make them particularly practicable.

Chlorids.—*Determination of Chlorids in the Urine (Seelman's[3] Modification of Volhard's Procedure).*—Principle: Precipitation of the chlorids as silver chlorid and titration of the excess silver nitrate after the precipitation. Procedure: Place 0.5 ml. of the urine in a small evaporating dish, using a Mohr 2-ml. pipette (Fig. 229) graduated in

[1] Compare O_2 content of bile, 0.39 vol. per cent. The data are from Buckmaster, G. A. and Hickman, H. R. B. Intern. Physiol. Courg. Stockholm, 1926, Abs. p. 28.

[2] Compare bile: 13.6 vol. per cent.

[3] Seelman, J. J., physician, Milwaukee, Wisconsin. See Jour. Lab. and Clin Med., vol. 1, p. 444, 1916. For solutions see Appendix.

hundredths of a ml. Pass a stream of water through the pipette and then distilled water. Drain it as free as possible from water. Pipette exactly 1 ml. of Seelman Solution I into the dish, using a 1-ml. Ostwald-Folin pipette. Stir the contents of the dish with a small solid glass stirring-rod. Now draw up in the clean 2-ml. Mohr pipette enough of Solution II to bring the meniscus above the starting-point of the readings; adjust the meniscus. Let drops flow from the pipette into the dish, stirring all the time, until the brownish color persists for at least ten seconds. Read the pipette.

Fig. 229.—Mohr type pipettes.

Calculation: Subtract from 2 mls. the reading of the pipette. This is automatically done by the type of pipette shown in Fig. 229; if you have a pipette graduated downward from 0.0 to 2.0 mls., the subtraction must be done by you. This gives the grams of NaCl per 100 mls. of urine.[1]

Comment.—The original Volhard method involved the separation of the silver chlorid from the solution containing the silver nitrate. This was advocated because some of the silver chlorid is converted into silver thiocyanate during titration and, consequently, more thiocyanate is used than the quantitative results demand. This gives higher figures than the theoretical one for chlorids in urine. However, in such minute quantities as are used in the above method, the error is about 5 mls. of thiocyanate, or only about one one-thousandth of the amount of sodium chlorid excreted in the urine per twenty-four hours.

Explanation of the Reactions.—Nitric acid is used in the solutions to prevent the purins of the urine from interfering with the reactions. They are precipitated as purin silver nitrate and utilize some of the

[1] Each 2 mls. of Solution II correspond to 1 ml. of Solution I and this corresponds to 0.01 g. of NaCl. Each ml. of Solution II corresponds to 0.005 g. of NaCl. If we designate the result (2 − n), being the titration figure, by y, then 5 y mgs. of NaCl occur in the 0.5 ml. of urine taken for the determination. For 100 mls. of urine we have 5 y (2 × 100) mgs. This is the same as 1000 y mgs., or y gs. per 100 mls. of urine (percentage).

thiocyanate added in titration, unless nitric acid be added. Iron alum (ferric ammonium sulphate, $Fe_2(SO_4)_3(NH_4)_2SO_4$) is added to act as an indicator; when all of the excess silver nitrate has been converted into silver thiocyanate (AgCNS, white), then ferric thiocyanate ($Fe_2(CNS)_6.(KCNS)_9$, brown),[1] appears owing to the reaction between the thiocyanate and ferric alum.

Volhard Method.[2]—Principle: The principle is the same as that of the Seelman modification. Larger amounts of urine and of reagents are used. The precipitated silver chlorid is removed by filtration. Procedure: Pipette 10 mls. of urine into a 100-ml. volumetric flask. Dilute with about 50 mls. of distilled water. Add 5 mls. of the indicator, ferric alum. Add 5 mls. of pure white nitric acid.[3] Add 20 mls. of standard silver nitrate solution. Dilute to the mark (100 mls.), stopper, invert, and mix. Filter through a dry filter-paper. When more than half has been filtered, pipette 50 mls. of the clear filtrate into a large evaporating dish and titrate with the thiocyanate solution. Calculation: One volume of the silver solution is equivalent to two volumes of the thiocyanate. Each ml. of the silver solution is equivalent to 8.23 mgs. of NaCl. Then:

$20 - n \times 8.23$ gives the mgs. per 10 mls. urine.
Multiply by 10 for per cent. chlorids as NaCl.

Phosphates.—*Free or Non-conjugated Phosphates in the Urine (Method of Bell and Doisy*[4]*).*—Principle: Ammonium molybdate solution is added to urine. Phosphomolybdic acid is formed. The phosphomolybdic acid is then caused to assume a color characteristic of the amount of phosphorus by reducing the phosphomolybdic acid. This is accomplished by means of hydrochinon acting in an alkaline medium.[5]

Procedure: Transfer 2 mls. of urine to a 100-ml. volumetric flask. Make the standard at the same time. Pipette 5 mls. of the standard

[1] Such compounds are called "rhodonates" from the Greek *r(h)odon*, a rose.

[2] Liebig's Annalen der Chemie, vol. 190, p. 1, 1878.

[3] Free from the lower oxids of nitrogen and, of course, from chlorids. Nitrous acid must not be present, because it forms a red compound with the thiocyanate, which leads the operator to believe that an end-point has been reached before the actual titration is completed.

[4] Washington University and St. Louis University, St. Louis, Mo., respectively, Jour. Biol. Chem., vol. 44, p. 55, 1920.

[5] The reaction is similar to that of the Folin method for uric acid (page 725). Ammonium molybdate $(NH_4)_6Mo_7O_{24}$ may be considered for this reaction simply as molybdic acid (molybdenum trioxid), MoO_3. When phosphoric acid (H_3PO_4) is added, phosphomolybdic acid ($H_3PO_4(MoO_3)_{12}$) is formed. This becomes reduced by hydrochinon (di-hydroxy-benzene, $C_6H_4(OH)_2$) to a blue molybdenum salt (see page 163).

KH_2PO_4 solution[1] into a similar 100-ml. volumetric flask. Now to each flask add 25 mls. of distilled water, 5 mls. of the special molybdic acid solution, and 5 mls. of the hydrochinon solution. Rotate the flasks to mix the contents and let stand for five minutes. Add 25 mls. of the special sulphite solution to each flask. Dilute to the mark with distilled water and, after mixing, let stand for five minutes. Pour, simultaneously into the two cups, liquid from each flask and insert the plungers of the colorimeter at the same time. Permit to stand two minutes. Then place the standard cup (left) at 20 mms. Adjust the right cup to such a depth that the color matches that of the left cup. Then take the reading.

Calculation:

$$\frac{\text{Reading of the standard (20)}}{\text{Reading of the unknown (n)}} = \frac{\text{Concentration of unknown (x)}}{\text{Concentration of standard (0.5)}}$$

Or:

$$x = \frac{10}{n} \text{ mgs. for 2 mls. of urine,}$$

or:

$$x = \frac{500}{n} \text{ mgs. per cent. of inorganic phosphate in urine.}$$

Sutton's[2] *Method for Non-conjugated Phosphates.*—Principle: The phosphate is precipitated as uranium phosphate by adding a known quantity of uranium salt (acetate or nitrate in acetate solution). The reaction is as follows:

$$BH_2PO_4 + \begin{matrix} H_3C.COO \\ \\ H_3C.COO \end{matrix} \!\!> UO_2 = CH_3.COO.B + CH_3COOH + UO_2H_2PO_4.$$

Phosphate like K- or Na-phosphate. | Uranium acetate. | An acetate. | Acetic acid. | Uranium phosphate (yellow).

An indicator is used to define the end-point of the titration. Usually potassium ferrocyanid is used, which becomes uranium ferrocyanid (brown) when the phosphate has all been precipitated. Since this gives a slightly high reading, cochineal[3] may be used.

Procedure: Pipette 50 mls. of the urine into a 400-ml. beaker. Add 5 mls. of the special sodium acetate solution[4] and then a few drops of cochineal solution to serve as an approximate indicator.

[1] Appendix. The solution is made in such a manner that 5 mls. contain 0.5 mg. phosphorus.

[2] Sutton, F., English analytical chemist. See Chem. News, vol. 1, pp. 97, 122, 1860. Also Sutton, F. (revised by W. L. Sutton and A. E. Johnson), A Systematic Handbook of Volumetric Analysis, Philadelphia, P. Blakiston's Son & Co., 11th ed., 1924. The Germans, Neubauer and Pincus, suggested the same procedure in 1907.

[3] Carminic acid, $C_{17}H_{18}O_{10}$. The end-point is indicated when the solution turns green.

[4] Appendix.

Heat the contents of the beaker to about 60° C.[1] From a burette, either held in the hand or clamped above the beaker, add standard uranium solution until the color changes from the red of the cochineal to green. Arrange a spot plate containing potassium ferrocyanid solution in the depressions and, with a stirring-rod, add a drop of the boiling solution to a drop of the ferrocyanid on the plate. The exact end-point is indicated when a permanent brown color remains after application of the stirring-rod.

Calculation: The solution of uranium salt is so made that 1 ml. corresponds to 5 mgs. of phosphate, estimated as P_2O_5. Then:

The number of mls. of uranium solution used in titration $\times$ 5 = milligrams of P_2O_5 in 50 mls. of urine.

Or:

This result, multiplied by 2, gives the milligrams percentage P_2O_5.

The twenty-four-hour quantity is found by taking 0.01 of the figure and multiplying by the number of mls. in twenty-four-hour volume.

Urinary Inorganic Phosphate; Method of Fiske and Subbarow.—Principle.—A blue color is produced by reduction of phosphomolybdic acid by means of a substance which acts on phosphomolybdic acid but not on molybdic acid. The phosphate as phosphoric acid reacts with molybdic acid to form phosphomolybdic acid. Procedure: Pipette 2 mls. urine into a 100 ml. volumetric flask. Similarly, make the standard by pipetting 5 mls. of KH_2PO_4 solution (containing 0.4 mg. phosphorus; 0.3510 g. KH_2PO_4 per liter) into a second 100 ml. volumetric flask. Dilute the contents of each flask up to about 70 mls. with dist. H_2O. Add, also, to each flask 10 mls. molybdic acid solution.[2] Lastly, to each flask add 4.0 mls. of the special reducing reagent for phosphates (page 922).[3] Dilute to the mark, mix, let stand 5 minutes and compare in a colorimeter. The standard is set at 20.0 mms.

Total phosphates (non-conjugated and those oxidized from conjugated phosphates):

[1] This is to insure that the calcium phosphate of the urine is not precipitated, as it readily is in acetic acid solution when boiled. After considerable uranium solution has been added, then the solution may be brought to the boil.

[2] Weigh, roughly 2.5 gs. ammonium molybdate into a 100 ml. volumetric flask. Fill to the mark with 5 normal H_2SO_4 solution.

[3] This reagent is used as a 1:1000 solution in bisulphite solution: Dissolve 0.5 g. of the reagent prepared on page 922 in 500 mls. of a sulphite solution made by weighing 25 gs. crystal sodium bisulphite or pyrosulphite (page 924) into a 500 ml. volumetric flask, adding 5 mls. of 20 per cent. sodium sulphite and diluting to the mark.

Method of Neumann.[1]—Principle: Phosphorus united to organic substances (nucleoprotids, lecithin, etc.) is hydrolyzed and oxidized to inorganic phosphate. Then the total phosphate (free and that released from organic compounds) is estimated by the above method. Procedure: Pipette 10 mls. of urine into a large Kjeldahl flask. Add 10 mls. of concentrated sulphuric acid and 5 mls. of concentrated nitric acid. Place the flask on the digestion shelf of the Kjeldahl apparatus in the hood and heat gently. When the colored fumes (oxides of nitrogen) are no longer given off from the solution, the residue should be white. If it remain colored, let the flask cool and continue cooling under the tap. Then add 3 mls. of nitric acid to the contents of the flask and heat again. Cool as before, add about 150 mls. of water and 20 mls. of ammonium nitrate solution (80 per cent.). Heat to 85° C. Permit to cool somewhat and then add 20 mls. of 10 per cent. solution of ammonium molybdate. Heat gently and note the formation of a yellow color. Shake at intervals for fifteen minutes. Let the solution cool during this time, and when at about room temperature filter off the precipitated phosphomolybdate.[2] Rinse the Kjeldahl flask with a small amount of cold water and add the rinsings to the residue on the paper. Then, finally, wash the residue with a small amount of cold water. Remove the crucible or funnel and transfer the contents to the flask, using distilled water to aid in solution. Add, also, enough half-normal NaOH solution to dissolve the precipitate, recording the amount of alkali used. Then add 10 mls. excess. Add about 300 mls. of distilled water. Now boil the solution in order to remove the ammonia. Cool the flask under the tap and titrate the excess of alkali which has been added, using 0.5 normal sulphuric acid, with phenolphthalein as indicator. Calculation: Each ml. of the alkali used to cause solution of the precipitate corresponds to 0.0012 g. P_2O_5. Then:

(mls. alkali added + 10 mls.) − (titration figure) × 0.0012
gives the number of gs. total phosphate as P_2O_5 per 10 mls. urine.
The result × 10 gives the percentage of P_2O_5.[3]

Sulphur in the Urine.—It is customary to consider four different quantities of sulphur in urine:

[1] Neumann, A. (German chemist). The method is given in Sutton, cited on page 791. See also Zeitschr. f. Physiol. Chemie, vol. 37, p. 129, 1902.

[2] Filtering is best accomplished by using suction and a good Gooch or alundum crucible. However, with care, filtering may be done through paper, although it is better to support the paper by means of a small perforated porcelain plate.

[3] If the result is to be expressed in terms of phosphorus, then the factor is 0.0005 g.

1. *Total Sulphates.*—All of the sulphur of urine is oxidized, during the process of making the determination, to sulphate, SO_4.

2. *Inorganic Sulphate.*—Sulphur having been oxidized in the body, particularly in the liver, to sulphate, SO_4.

3. *Ethereal Sulphates.*—Conjugated SO_4 with compounds like the products of putrefaction, phenol, indoxyl, etc. The linkage between the radicle SO_4 and the compound is an oxygen, and this is the basis of the term "ethereal."

4. *Neutral sulphur*, in the form of "acid" or sulph-hydryl sulphur, SH. This represents the sulphur of the body which has not been oxidized. It is composed partly of food compounds containing sulphur, that have not been oxidized in the liver to SO_4; also of the sulphur from metabolized protids that escapes oxidation in the liver. Inasmuch, however, as neutral sulphur varies directly with the metabolism of the body, exogenous compounds containing sulphur probably play only a subordinate part in its formation.

Methods of Determining Total Sulphates.—*Method of Rosenheim and Drummond.*[1]—Principle:[2] Benzidin hydrochlorid is a weak organic base, neutral to phenolphthalein. It may act as a bearer of an acid; with sulphuric acid, a salt, $C_{12}H_8(NH_2)_2.H_2SO_4$, insoluble in water slightly acidulated with HCl. The sulphuric acid is easily removed from the benzidin when the solution is titrated with a caustic alkali, like NaOH.

Procedure: For total sulphates (method modified by use of Benedict's reagent): Principle: The neutral sulphur, SH, is oxidized by means of $KClO_3$ and $Cu(NO_3)_2$ to inorganic sulphate, SO_4. Then the sulphate is determined by the benzidin method. Method: Pipette 10 mls. of urine into an evaporating dish. Add 5 mls. of the special Benedict reagent for oxidizing sulphur.[3] Leave the dish on the steam-bath until the liquid has evaporated. Then cover it with a large watchglass and place it on a tripod over a moderate flame. Heat for about five minutes until the cupric nitrate of the reagent has

[1] English chemists; see Biochem. Jour., vol. 8, p. 134, 1913. The use of benzidin for this purpose was first suggested by Mueller (German chemist) in 1902. Besides the authors mentioned, similar methods have been suggested by Raiziss and Dubin (Dermatological Laboratory, Philadelphia), by Hibbard (soil biochemist of the United States), and by others. Rosenheim and Drummond have suggested a routine for the analysis of different fractions of sulphates in the urine.

[2] For a discussion of the principle of the use of benzidin in sulphate determination see Treadwell, F. P. (tr. by Hall, W. T.), Analytical Chemistry, vol. ii, p. 714, 5th ed., 1919, New York, John Wiley & Sons. Also Sutton (cited on page 791), p. 340.

[3] Appendix.

become oxidized to cupric oxid. Let the dish cool. Add 10 mls. 1 : 10 HCl solution and also about 10 mls. of water. Aid the solution of the residue with a "policeman." Neutralize the solution, using 10 per cent. NaOH solution and litmus-paper. Add, in addition, enough of the alkali to render the solution slightly alkaline. Place the dish on the steam-bath again in order to warm it. Filter off the cupric oxid by means of suction. Wash with distilled water. Reject the residue. To the filtrate add about 2 mls. of concentrated HCl and 100 mls. of benzidin hydrochlorid solution.[1] The benzidin sulphate thus formed appears as an evident precipitate. Filter through a paper supported by a porcelain plate. Wash with water that is saturated with benzidin sulphate solution. Transfer the residue and its paper to a beaker. Add about 50 mls. of distilled water and heat to 80° C. Add about 4 drops of phenolphthalein as indicator. Titrate the solution with decinormal sodium hydroxid solution until it becomes red. Calculation: Each ml. of decinormal NaOH is equivalent to 1 ml. of 0.1 normal H_2SO_4. The weight of sulphuric acid in 1 ml. of decinormal acid solution is 0.0049 g. Then:

Titration figure × 0.0049 = gs. H_2SO_4 per 10 mls. of urine.
Multiplied by 10, we obtain per cent. SO_4.

For Inorganic Sulphates.—Pipette 25 mls. of urine into a 250-ml. Erlenmeyer flask. Add 2 mls. of hydrochloric solution 1:3 and 100 mls. of the benzidin solution used above. Agitate the contents of the flask and let stand for about ten minutes. Filter.[2] Add 10 mls. of the benzidin sulphate solution to the flask, rinse it, and add the rinsings to the residue on the filter. Now transfer the filter-paper and residue to the flask, rinsing all parts that have come into contact with the solutions into the flask. Break up any aggregates of benzidin sulphate by means of a "policeman." Then heat the solution almost to the boiling-point and titrate with decinormal NaOH solution, using phenolphthalein as indicator.

Calculation: This may be carried out exactly as in the case of total sulphates, or, if it is desired to express the result in terms of SO_3, 1 ml. of decinormal NaOH is equivalent to 4 mgs. of SO_3. Then the neutral sulphur is obtained by subtracting the quantity thus obtained from that of the total sulphates.

Ethereal Sulphates. By subtracting the figures for inorganic sulphates from those for total sulphates, the amount of ethereal sulphates, expressed as either H_2SO_4 or SO_3, is obtained.

[1] Appendix.

[2] The filtrate must be passed through the filter again in case it is not entirely clear.

Unoxidized Sulphur, SH-sulphur or "neutral sulphur." This fraction may be obtained by using the data acquired from the determination of inorganic sulphates, together with that determined by the following procedure for inorganic and ethereal sulphates. The principle involves the acid hydrolysis of the ethereal sulphates and their determination along with the inorganic sulphates. Procedure: Pipette 25 mls. of urine into an Erlenmeyer flask. Add 20 mls. of 1:5 hydrochloric acid solution and boil half an hour. Cool, neutralize with 10 per cent. NaOH solution (litmus-paper), then reacidify with the HCl solution until Congo-red paper is turned from red to blue. Add 100 mls. of the benzidin hydrochlorid solution. Agitate the contents and leave for fifteen minutes. Filter. Wash the flask with about 10 mls. of saturated benzidin sulphate solution and add the washings to the residue on the paper. Reject the filtrate and add the residue and paper to the flask. Warm to about 100° C. Titrate with 0.1 normal NaOH solution with phenolphthalein as indicator. Calculation: The calculation is exactly similar to that of the preceding determinations: 1 ml. of 0.1 normal NaOH solution is equivalent to 0.0049 g. H_2SO_4, or to 0.004 g. SO_3. Sodium, potassium, and calcium may be determined gravimetrically. Such data are desirable in studies of acid-base balance in the organism. Since we have methods for determining total base and total acid excretion, it is seldom necessary to resort to the determination of these individual substances. The reader may consult the following references for methods of determining sodium, potassium, calcium, and magnesium:

Fiske's Sulphur Methods.—I. Removal of the phosphate for determination: Pipette, accurately, 25 mls. of mixed urine into a 100 ml. volumetric flask. Add one volume of dist. H_2O. Add 1 drop of indicator phenolphthalein. Then add, drop by drop, ammonium hydroxid to a beginning pink color. Add, in addition, 10 mls. of a 5 per cent. ammonium chlorid solution and finally, roughly 1.5 g. of powdered magnesium carbonate ("basic"). Make to the mark with dist. H_2O, invert the flask several times and then leave for 30 minutes. Filter, quantitatively. This filtrate is used in the following methods: II. *Inorganic Sulphate.*—Principle: Benzidin is used as in the method on page 795. Procedure: Using the filtrate from (I), transfer, by means of a pipette, 5 mls. to a 200 × 20 Pyrex test-tube. Add 2 drops of brom-phenol-blue (page 917) and 5 mls. dist. H_2O. Follow these additions with normal solution HCl, drop by drop until the blue color gives way to a distinct yellow. Now add 2 mls. of

the benzidin reagent[1] and leave 2 minutes. Lastly, add 4 mls. 95 per cent. aceton and leave at least 10 minutes. Preparation of filter: In the bottom of a Fiske tube (see Fig. 230) prepare a very thin mat of paper pulp. Insert the stopper through which the tube passes, into your filter-flask, add 10 mls. distilled water and suck dry by means of your filter-pump. Now before vacuum is established, transfer the benzidin sulphate solution to the filter-tube, using not over 1 ml. of 95 per cent. aceton for washing down the sides of the larger tube. Use gentle suction. When dry, rinse the larger tube again with 1 ml. of aceton and finally, with 5 mls. Remove the filter tube. By means of a wire, force the precipitate and its mat through the hole of the filter-tube into the 200 × 20 mm. tube first used. Rinse any adhering precipitate from the wire by means of a small amount of water from your water-bottle. Heat the contents of the tube to about 60° C. and while hot, titrate with 0.02 normal NaOH solution, phenol red as indicator. Calculation: Since benzidin holds $SO_4^=$ very lightly, we may consider that we are titrating a solution of H_2SO_4 with an alkali. Multiply the titration figure by 0.32 to find the milligrams of inorganic sulphur in the 5 mls. of filtrate. To find per cent., multiply titration figure by 25.6.

Determination of *sodium* and *potassium:* Folin's manual.

Determination of *calcium* and *magnesium:* The method is given in Folin's manual, but the original description was made by McCrudden.[2]

Iron may be determined by the method of Wolter.[3] The description of the method is given by Hawk. Fowweather[4] gives the amount of iron in urine as 0.04 mg. per 100 mls. urine.

Iodin occurs in small quantities. The determination in the urine is sometimes made following administration of iodids.

Method of Marsh.[5]—Principle: Total halogen is determined by the Volhard method[6] and in a second sample, after the iodin has been freed from the mixture of halogens by nitrous acid.[7] Procedure:

[1] Rub up in a mortar 4 gs. benzidin with enough dist. H_2O to make a paste. Then transfer to a 250 ml. volumetric flask by means of about 150 mls. H_2O. Add 50 mls. normal HCl solution. Mix dilute to the mark and filter until clear.

[2] McCrudden, F. H. (Professor of Applied Therapeutics, Tuft's Medical School, Boston, Mass.), Jour. Biol. Chem., vol. 7, p. 82, and vol. 10, p. 187, 1911.

[3] Wolter, M. (German chemist), Biochem. Zeitschr., vol. 24, p. 103, 1910.

[4] Fowweather, F. S., Univ. Leeds. Biochem. Journ., vol. 20, page 93, 1926.

[5] Marsh, H. L. (University of Illinois College of Medicine, Chicago). See Jour. Lab. and Clin. Med., vol. 8, p. 271, 1923.

[6] Page 790.

[7] See Treadwell-Hall, cited on page 794, p. 331.

Pipette 50 mls. of urine into an evaporating dish, preferably of nickel. Add 35 mls. of fusion mixture solution[1] and leave on a tripod over a low flame until a dry residue is left. Then turn up the flame and fuse. When the blackness of carbon has disappeared, cool, and add 100 mls. of distilled water. Care must be taken throughout that no liquid is lost by spattering. The dish should be kept covered with a large watch-glass. Next a determination for total halogen is made according to the method of Volhard, given on page 790. Fifty mls. of the filtrate, after precipitating the halogens as silver salts and filtering, are used for the determination. Now proceed to the determination of

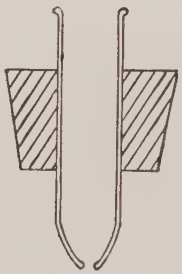


Fig. 230.—Fiske filtration tube (one-half natural size). (From Journal of Biological Chemistry, June, 1921).

iodin-free solution. Follow the method given above to the completion of the fusion. Then transfer the 100 mls. of solution to a 2-liter flask. Add about 1000 mls. of water. Add 3 mls. of concentrated chlorid-free H_2SO_4 and 10 mls. of a special nitrous acid solution.[2] Boil the solution until all the iodin has been evolved. The solution becomes colorless. This takes about half an hour, but may be continued longer. The determination is made by the Volhard method: Add to the cooled solution 110 mls. of decinormal silver nitrate solution in order to prevent the volatilizing of chlorin during the following concentration. Now concentrate the solution to 200 mls.[3] Transfer the residual fluid quantitatively to a 250-ml. volumetric flask and make up to the mark with distilled water. Make Volhard determination as before. Calculation: The iodin quantity is determined by subtracting the data found by the second determination from the amount in the first determination.

[1] Four grams per cent. of NaOH and 20 gs. per cent. $NaNO_3$, aqueous.

[2] Appendix.

[3] This process of concentrating may be done in a large evaporating dish, but spattering must be avoided. "Bumping" may occur in the flask.

Method of McClendon.[1]—The principle of this method is a micro-colorimetric determination of iodin dissolved in carbon tetrachlorid. The method, designed for iodin in water, is applicable to the urine. In the case of albuminuria the protid must be removed.

Total Base in the Urine.—In evaluating the acid-base relations of the body it is necessary to know the potential basicity of the substances in the urine. Excellent methods have been suggested for this purpose. Fiske's method is given herewith:

Method for Fixed Base of Fiske.[2]—Principle: Conversion of the base into sulphate and the determination of the sulphate by the benzidin method. Procedure: Pipette 5 mls.[3] of urine into a 200 × 20 mm. test-tube of resistance glass, like Pyrex. Add 1 ml. of approximately 4-normal[4] sulphuric acid, 0.5 ml. concentrated nitric acid, and a quartz piece to prevent bumping. Boil over a micro-burner until white fumes[5] appear. The residue in the bottom of the tube should become colorless about this time. In case it does not, let the preparation cool somewhat, then add 4 drops of nitric acid and continue the heating. Remove the flame, let the tube cool for about three minutes, and then carefully wash the contents of the tube into a urea-tube graduated at 25 mls., using not more than 10 mls. of distilled water for the washing. Add a drop of methyl-red indicator solution and then solid ammonium carbonate until the color begins to change. Next add enough 4-normal H_2SO_4, drop by drop, to restore the pink color. Heat to boiling and, if necessary, again restore the pink color by means of the acid solution. Add about 0.5 ml. of the special ferric chlorid solution[6] and agitate the contents of the tube. Then add 1 ml. of 5 per cent. ammonium acetate solution and enough distilled water to make a total volume of about 10 mls. Heat to boiling, dilute to the mark (25 mls.), using cold distilled water, close the mouth of the tube with a rubber stopper, invert

[1] McClendon, J. F. (University of Minnesota, Minneapolis, Minn.), Jour. Biol. Chem., vol. 60, p. 289, 1924.

[2] Fiske, C. H. (Assistant Professor of Biological Chemistry, Harvard Medical School, Boston, Mass.), Jour. Biol. Chem., vol. 51, p. 55, 1922.

[3] The amount of urine should be such that not over 0.005 mg. of inorganic phosphate is involved. This may be found by the method given on page 792. If the urine contain more than 5 mgs. of inorganic phosphate per 5 mls., reduce the number of mls. taken for observation. Unless there is a kidney involvement, causing retention of phosphate, it is never necessary to increase the amount of urine used in the determination. On the other hand, if the urine is considerably concentrated, one must use less of it.

[4] This is about 20 per cent. of the desk reagent.

[5] White fumes must be distinguished from those of partly condensed water and other vapors.

[6] Appendix.

several times, and pour the contents of the tube upon a dry, ashless filter-paper in a small funnel. Collect about 20 mls. of the filtrate in a test-tube. Stopper the tube and hold it under the cold tap until the contents are cool. Transfer 5 mls. of the filtrate to a small evaporating dish.[1] Add 1 ml. of the special 4-normal H_2SO_4 and evaporate on a water-bath until nearly dry. Then lay the dish on a pipe-stem triangle and apply heat from the standard Bunsen burner. Keep the flame low to begin with, raising it gradually, until all the fumes cease to come off. Remove the flame and let the dish cool. Then sprinkle over the residue in the dish some powdered ammonium carbonate and heat again. Raise the flame to the maximum and continue heating while the dish is red. Each part of the dish must receive this treatment and it is best to move it about the triangle by means of crucible tongs. Then remove the flame and let the dish cool. Add about 2 mls. of water and dissolve the residue, using a "policeman." Wash off the policeman with a few drops of water from your wash bottle. Now wash the contents of the dish quantitatively into a 200 × 20 mm. test-tube of resistance glass, using about 2 mls. of water for each of four washings. Determine the inorganic sulphate content of the test-tube by the benzidin method,[2] using 0.02 normal NaOH for the titration of the benzidin.

Calculation· The number of mls. of 0.02 normal NaOH solution used in the titration less 0.01 (temperature correction) gives the fixed base in terms of decinormal solution for the number of mls. of urine taken. In the above case 5 mls. of urine were taken. The per cent. base then is 20 times the result obtained.

Total Base May Be Determined by Other Methods, Notably by That of Greenwald[3] *and That of Sumner.*[4]—The method of Greenwald is based upon the following principle: The bases of the urine are combined with anions like $Cl,^-$, $SO_4^=$, $H_2PO_4^-$, and HCO_3^-, and, in the case of bases excreted in albuminuria of nephrosis, with protid.[5] If we add an excess of acid (HA) of the proper kind, in excess of that needed to precipitate anions, any protid is precipitated as a combination with the acid and the bases are salts like BA, BH_2PO_4, etc.

[1] Dishes of platinum or platinum substitute are preferable, but those of nickel or of porcelain may be used; Pyrex or Jena glass dishes are desirable.

[2] Page 796.

[3] Greenwald, I. (page 23), Jour. Biol. Chem., vol. 54, p. 263, 1922. The method as published is adapted to determinations in blood, but the procedure may readily be adapted to the analysis of the urine.

[4] Sumner, J. B. (page 24).

[5] See page 108 concerning the existence of protid as anion.

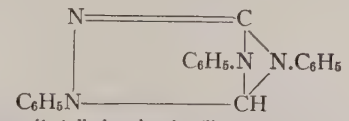
Then, if we determine the excess acid remaining after precipitation and also the amount of the anion of the acid that is used in precipitating the protid of the blood, the difference represents the amount of anion of the acid that is used in precipitating the bases of the blood. The excess acid is determined by titrating with an alkali and suitable indicator; the anion of the acid in the present case is picrate, since picric acid is used as the acid-precipitating agent. The amount

Fig. 231.—Tyrosin crystals from urine (× 450). (From Todd, Clinical Diagnosis by Laboratory Methods.)

of picrate is determined by one of two methods: (1) Gravimetrically as nitron picrate;[1] (2) volumetrically, using titanous chlorid, $TiCl_3$.[2]

Sumner's method employs di-nitro-salicylic acid[3] in place of picric acid. As in the case of picric acid, dinitrosalicylic acid precipitates the protids. Likewise, it is possible to determine, colorimetrically, the excess dinitrosalicylic acid and the free and combined fraction,

[1] Nitron is a modified pyrrol (see hemoglobin, page 383) compound; strongly basic in nature, but easily split into the original substances when it is made into a salt like nitron picrate. Nitron is:



Nitron (1, 4-di-phenyl-end-anilino-dihydro-triazol).

The nitrate, picrate, etc., are very insoluble, which is a quality of special advantage in this determination.

[2] See Knecht, E., and Hibbert, E. (German and English chemists), New Reduction Methods in Volumetric Analysis, New York, Longmans, Green & Co., 1910.

[3] Page 233. The method is published in Jour. Biol. Chem., vol. 56, p. 701, 1923.

thus making it easy to obtain the combined fraction by difference. Dinitrosalicylic acid in the presence of ferric chlorid becomes very red in color. In the procedure given below the amounts of reagents are designed for blood bases, but by suitable dilution of the urine the

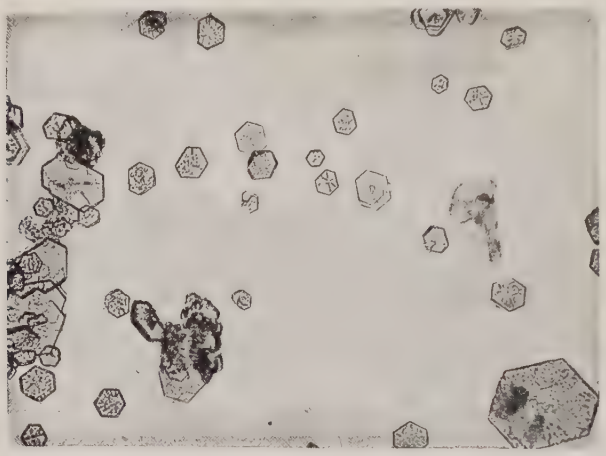


Fig. 232.—Cystin crystals from urine of patient with cystin calculus (× 200). (From Todd, Clinical Diagnosis by Laboratory Methods.)

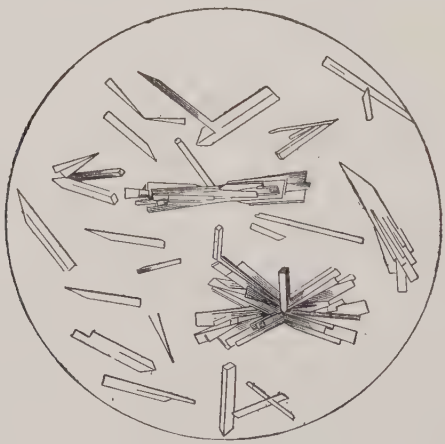


Fig. 233.—Stellar calcium phosphate. (From Holland, Medical Chemistry and Toxicology.)

method is applicable directly to that fluid. Procedure: (1) Precipitation: Two mls. of oxalated blood, diluted with 8 mls. water are treated with 10 mls. of the reagent.[1] Filter. (2) Free acid: Heat 5 mls. of the filtrate to boiling; cool; titrate with 0.01 n. alkali

[1] Appendix.

(methyl-red indicator, then with thymolphthalein indicator). (3) Free and combined acid: Make colorimetric comparison of a solu-

Fig. 234.—Triple phosphate in urine and spheres of ammonium urate. (From Holland, Medical Chemistry and Toxicology.)

Fig. 235.—Calcium oxalate. (From Holland, Medical Chemistry and Toxicology.)

tion of 5 mls. filtrate treated with 5 mls. 10 per cent. ferric chlorid and made up to 100 mls. with H_2O, with standard. Calculation:

$$\text{Free + combined acid} = \frac{R_s}{R_u} \times \text{mls. 0.01 normal alkali used to neutralize 0.5 ml. acid.}$$

$$\text{Titrable alkali in 0.5 ml. blood expressed as 0.01 normal} = \text{Quantity just determined minus} \begin{cases} \text{Mls. 0.01 n alkali neutralizing 5 mls. filtrate to methyl-red indicator.} \end{cases}$$

$$\text{Titrable alkali in 0.5 ml. blood} \times 20 = \begin{cases} \text{Titrable alkali in 100 mls. of blood expressed as decinormal.} \end{cases}$$

Calcium.– Nearly all calcium (95–98 per cent.) is excreted in the feces. On low Ca intake, more is excreted than ingested. Urinary calcium has no clinical significance nor does it play an important function in metabolism studies. During fasting, during which little fecal matter is passed, an increase of urinary Ca occurs up to a constant quantity,[1] which probably represents that endogenously metabolized. During periods of low Ca intake, teeth and bone may become decalcified to furnish metabolic Ca. It has recently been shown that calcium is retained in pregnant women who are subjected to ultraviolet radiations.

SUMMARY

1. The urine is one of the means of excretion.
2. It is the chief and, for practical purposes, the sole excretory avenue for nitrogenous end-products.
3. For this reason the study of the quantitative excretion of such substances in the urine is of great importance in discussions of metabolism.
4. Although in a given individual under controlled conditions the excretion of substances in the urine is fairly uniform, yet it varies with different physiological and pathological states.
5. Methods have been described by which accurate determinations of minute amounts of the various substances excreted in the urine may be made.
6. The methods outlined in this chapter will be used in the Practical Exercises in Chapter XVII.

SUGGESTED READINGS

Throughout this Chapter references have been made to special books and pamphlets. The more general publications on urinary excretion listed below serve to supplement the discussions just completed:

Barger, G. The Simpler Natural Bases, New York, Longmans, Green & Co., 1914.

Beaumont, G. E., and Dodds, E. C. (Professor of Biochemistry, University of London, England). Recent Advances in Medicine, Philadelphia, P. Blakiston's Son & Co., 3rd ed., 1927.

Cole, S. R. Practical Physiological Chemistry, Baltimore, Williams & Wilkins (American edition), 1925.

Cushny, A. R. The Secretion of the Urine, New York, Longmans, Green & Co., 1917.

Folin, O. Laboratory Manual of Biological Chemistry, New York, D. Appleton & Co., 4th ed., 1925.

[1] According to Benedict, a fall begins on the 20th day (1st day 0.217 g. urinary Ca; 18th day 251 gs.; 20th, 0.237 g.; 22nd day, 0.179 g.; 28th day, 0.131 g.). See Benedict, F. G. A prolonged study of fasting. Washington, D. C., Carnegie Institution of Washington No. 203, page 247, 1915.

Hawk, P. B. Practical Physiological Chemistry, Philadelphia, P. Blakiston's Son & Co., 1926.

Jones, W. The Nucleïc Acids, New York, Longmans, Green & Co., 1925.

Macleod, J. J. R. Physiology and Biochemistry in Modern Medicine, St. Louis, Missouri, C. V. Mosby & Co., 5th ed., 1926.

Neuberg, C. Der Harn. Jena, Gustav Fischer, 2 volumes, 1913.

Plimmer, R. H. A. Practical Organic and Biochemistry, New York, Longmans, Green & Co., 5th ed., 1926.

Rose, W. C. Purin Metabolism, Physiolog. Revs., vol. 3, p. 544, 1923.

Sherwin, C. P. The Fate of Foreign Organic Compounds in the Animal Body, Physiolog. Revs., vol. 2, p. 238, 1922.

Underhill, F. P. Manual of Selected Biochemical Methods, New York, John Wiley & Sons, Inc., 1921.

Webster, R. W. Diagnostic Methods, Philadelphia, F. Blakiston's Son & Co., 1923.

Werner, E. A. Urea, New York, Longmans, Green & Co., 1923.

CHAPTER XVI

METHODS FOR THE DETERMINATION OF BLOOD CONSTITUENTS

"Neither from a theoretical nor practical point of view can one concur in the assertion that pathological chemistry is separate and different from physiological chemistry."
—*Lehmann*, Physiological Chemistry, 1855.

In Chapter VII we discussed the blood as a tissue. In the present Chapter we shall consider it as an avenue of transportation for materials passing from the receiving system, the alimentary tract, to the consumers, the tissues, and for waste-matters passing from them to the discharging system, the kidneys. In this instance we shall be less interested in what the blood is than in what transport material it contains.

Fig. 236.—Victor C. Myers, Professor of Biochemistry, Western Reserve University, Cleveland Ohio. Contributor to the chemistry and metabolism of urinary and blood constituents.

In clinical diagnosis the data obtained from accurate studies of the urine have been of great value. However, it is difficult to collect urine with the accuracy essential to critical determinations; the volume varies considerably from hour to hour and dependable data can be obtained only when urine is collected accurately over prolonged periods, the standard interval being twenty-four hours. For such reasons, if for no other (and there are many more), the study of blood is greatly to be preferred. Blood is readily obtained at any period desired; its physical characteristics do not vary as widely as those of the urine; and variations in the blood constituents are far more characteristic of physiological and pathological states than those of urine.

In general, 10 mls. of blood is ample from which to ascertain a variety of data on organic and inorganic, as well as gaseous materials such as oxygen and carbon dioxid. This amount of blood is obtained by venous puncture with a hypodermic needle, without discomfort to the patient. In the young the blood is taken from the heel or from

Fig. 237.—Vacuum tube used for the collection of blood for analysis. The small test-tube above fits over the sterile hypodermic needle which is attached to the bulb by means of a rubber tube. The bulb is exhausted. When ready for use, remove the test-tube, insert the needle into the blood-vessel, break the glass tube passing through the rubber tube, and the blood will flow into the bulb.

the superior longitudinal sinus. The procedure is routinely used in clinical diagnosis. The layman is apt to think that this entails risk of infection, or of causing the formation of clots, which act as emboli, but such is not the case. The author has known, personally, of thousands of venipunctures, and in no case has there been any unfavorable development attributable to taking the sample.[1] On the other hand, in many of these cases, the subject has been saved from prolonged

[1] The only two instances that may have fallen under this category were (1) an air embolism in the immediate region of the puncture, made by careless operator, and (2) extensive extravasation in a case of an hemophiliac. One patient was punctured for over forty times and no more evidence of the operation after the last puncture was seen than would have followed the single insertion of the needle.

illness or even from death by the diagnosis made possible by blood analysis.

Wholly aside from the practical usefulness of blood chemistry, it is a boon to the sciences of physiology, pharmacology, and biochemistry, for without the accurate knowledge of the substances in the

Fig. 238.—Methods for securing blood by puncture of a vein. The middle figure shows distention of the veins of the arm about the elbow. The needle is entered by a quick upward thrust. Practically any prominent and firm vein may be used. The upper left-hand figure shows collection of blood in a test-tube. Usually 10 mls. or more are easily collected before clotting occurs. The lower right-hand figure shows collection of blood in a Keidel vacuum tube. (From Kolmer, Infection, Immunity, and Biologic Therapy.)

blood-stream, an understanding of many events in these departments would be impossible.

The following pages are devoted to the presentation of methods of blood analysis and the interpretation and clinical application of the findings.

NON-PROTID NITROGEN

The blood, like all tissues, contains protid, but, with the exception of certain instances to be discussed, the content of protid in the blood is of little or no significance either in pure biochemical studies or in clinical diagnosis. Consequently, the protids of the blood are rejected before determinations of the various constituents, organic or inorganic, are made. Whole blood is rarely used in the analyses. When protid has been removed from the blood the sum total of the nitrogen derived from the remaining nitrogenous substances as determined from the filtrate is spoken of as non-protid nitrogen. We cannot say, however, that non-protid nitrogen is the sum of the nitrogen belonging to the known substances like urea, etc., for experi-

Fig. 239.—Folin's digestion tube for total nitrogen determinations (non-protid nitrogen), blood urea, etc.

ments have shown that there is some nitrogen which belongs to substances of unknown nature. We may, therefore, more accurately define non-protid nitrogen as the total nitrogen of the blood less that of the protids. The total nitrogen of the blood is about 2.5 gs. per 100 mls. of whole blood. The non-protid nitrogen is about 1 per cent. of that amount, or 25 mgs. per 100 mls. of blood.[1] The undetermined nitrogen makes up about 3.6 per cent. of the non-protid nitrogen. The undetermined nitrogen is believed to consist of amines, histons, poly peptids, and nucleotids. It is present to greater extent

[1] In Benedict's laboratory normal non-protid nitrogen is found to be 28 to 35 mgs. per 100 mls. blood. See also Berglund, H., Jour. Amer. Med. Assoc., vol. 79, p. 1375, 1922. For the average normal non-protid nitrogen figure adopted in the author's laboratory see back cover of this book.

in corpuscles than in plasma, and therefore appears only when the corpuscles are hemolyzed. The plasma of the blood is of the nature of intercellular material, and one would naturally expect to find a

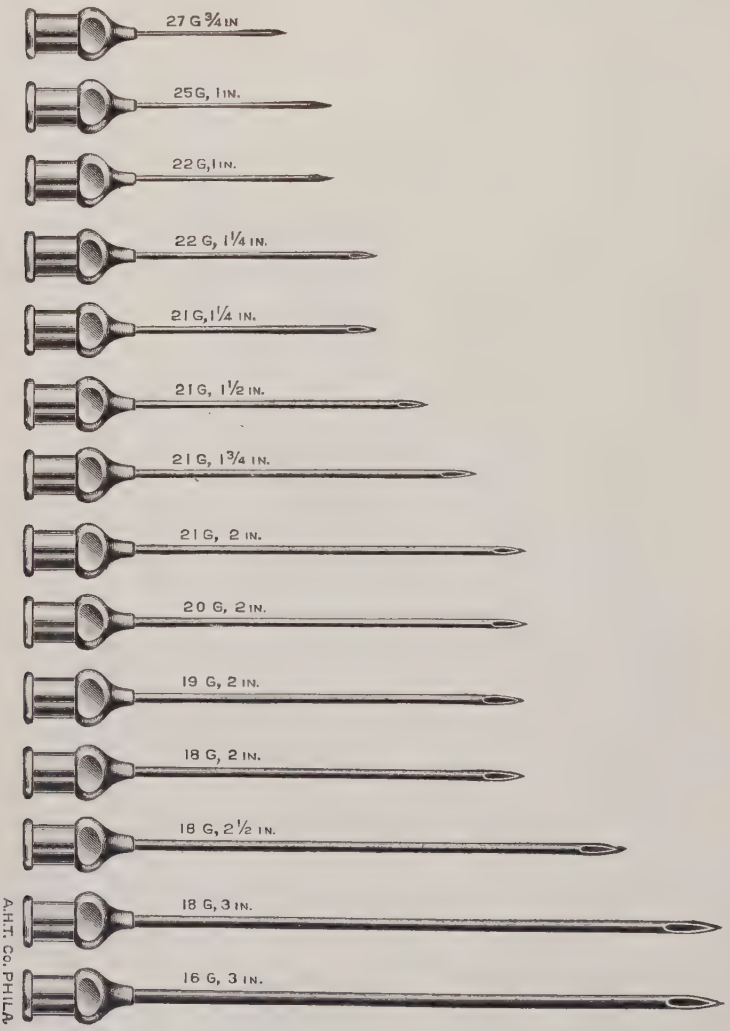


Fig. 240.—Sizes and shapes of hypodermic needles used in biochemical work. For the proper shape of the point see Fig. 246. Natural size.

greater quantity of nitrogen in the actively metabolizing corpuscles of the blood than in the passive plasma. When the plasma and the corpuscles are analyzed separately for nitrogenous substances this is found to be true.

	Mgs.
Total N. P. N.[1] in 100 gs. of plasma	24.7
Total N. P. N. in 100 mls. of corpuscles	43.6[2]

The non-protid nitrogen varies physiologically:[3]

1. Diet:

	Mgs.
Fasting	32.1
Glucid diet[4]	21.0
Low protid diet	32.0
High protid diet[5]	34.0

2. Time of day respecting meals:

	Mgs.
Control, from carotid artery before injecting	35[6]
80 minutes after injecting 5 gs. glycin into vein	130
Before injecting asparagin	43
22 minutes after injecting 10 gs. asparagin; blood sample from jugular vein	49
24 minutes after injecting the asparagin; blood sample from portal	69
47 minutes after injecting the asparagin; blood from carotid	52
120 minutes after injecting the asparagin; carotid	62
123 minutes after injecting the asparagin; portal	70
129 minutes after injecting the asparagin; mesentery	62
132 minutes after injecting the asparagin; inferior vena cava	62

3. Source of specimen: The difference in concentration of non-protid nitrogen in blood taken at different places in the body after meals is seen in the foregoing table 2, above, for, although the food (glycin and asparagin) was introduced intravenously and not through the usual channel, the mouth, the result is the same. However, during fasting there is a uniform distribution of non-protid nitrogen:

	Mgs.
N. P. N. portal blood	40[7]
N. P. N. of carotid	38

[1] The abbreviation "N. P. N." stands for non-protid nitrogen.

[2] After Folin, O., Non-protein Nitrogen of Blood in Health and Disease, Physiol. Revs., vol. 2, p. 460, 1922. The table is given as a whole on page 464 of Folin's article and is reproduced in the present book on pages 811–814.

[3] Gettler, A. O., and Baker, W., Jour. Biol. Chem., vol. 25, p. 211, 1916.

[4] Glucids probably act in this case as protid sparers. There is less mobilization of nitrogen in the blood. For protid-sparing action see page 566.

[5] This diet consisted of nearly twice the amount of protid normally eaten by the average man.

[6] The data were taken from Fiske, C. H., and Sumner, J. B., Jour. Biol. Chem., vol. 18, p. 285, 1914.

[7] Folin, O., and Denis, W., Jour. Biol. Chem., vol. 11, p. 87, 1912.

4. Exercise, rest, and other physiological states do not produce characteristic changes in the N. P. N. of the blood

The non-protid nitrogen varies in pathological states:

Normal or subnormal:

	Mgs.
Pregnancy:	
Case 1	25
Case 2	24
Case 3	22
Eclampsia, after convulsions:	
Case 1	26
Case 2	27
Case 3	23[1]
Prostate obstruction:[1]	
Case 1	20
Case 2	22
Case 3	24
Diabetes mellitus:	
Case 1	24
Case 2	24
Case 3	26
Cancer, uterine, very malignant[2]	22.4
Breast, rapid growth, male	22.4
Mouth, long duration, advanced	28.0
Sarcoma, prostate	23.8

[1] These figures may be higher in some cases.

[2] Theis, R. C., and Stone, W. S. (Cornell University Medical College, New York N. Y.), Jour. Biol. Chem., vol. 4, p. 349, 1919.

Above normal (retention):

	Mgs.
Incipient nephritis:	
Case 1	43.8
Case 2	45.4
Case 3	39.7
Nephritis, chronic.	
Case 1	166.6
Case 2	115.4
Case 3	139.2
Arteriosclerosis affecting kidney:	
Case 1	47.9
Case 2	39.1
Case 3	46.1
Cancer (deep):	
Case 1	32.0
Case 2	41.0
Case 3	37.8

Gout, during attack:	
Case 1	34.2
Case 2	25.5[1]
Nephritis, terminal	100 to 300
Anuria in bichlorid poisoning	400[2]
Anuria of prostate	22 to 120
Prostate obstruction, after prostatectomy	40 to 60
Intestinal obstruction, peritonitis, etc	50 to 60[3]

[1] Plasma, not whole blood; figures from Folin, O., et al., Jour. Biol. Chem., vol. 60, p. 361, 1924. They approximate those for whole blood (see Berglund, H., Jour. Amer. Med. Assoc., vol. 79, p. 1375, 1922.

[2] Case in Jefferson Hospital, 1923. Looney found a maximum (at death) of 214 mgs. N. P. N. per 100 mls. whole blood in a case of bichlorid poisoning. See Journ. Biol. Chem., vol. 70, page 512, 1926.

[3] Probably due to proteose toxicity, the proteose being absorbed from the intestine into the blood. See Jour. Amer. Med. Assoc., vol. 84, p. 122, 1925.

The manner in which the components of the N. P. N. vary in disease is shown in the following table (after Berglund):[1]

	Whole blood.					
	Amino-acid.	Urea.	Creatinin.	Uric acid.	Undetermined.	Non-protid.
Case 1	5.0	13	1.8	3.7	19.0	39
Case 2	6.4	49	2.0	4.5	7.4	65
Case 3	4.5	49	3.0	4.7	22.0	78
Case 4	5.8	60	7.7	4.8	37.0	107
Case 5	7.6	91	7.2	6.4	41.0	144
Case 6	8.4	174	12.9	13.6	75.0	267
Case 7	7.3	193	16.0	12.0	48.0	258

The retention of various substances making up the N. P. N. is fairly uniform. The variations between whole blood and plasma may be seen by comparing the table just given with the following, which is based upon the same cases:

	Amino-acid.	Urea.	Creatinin.	Uric acid.	Undetermined.	N. P. N.
Case 1	4.3	13	1.8	3.7	20	39
Case 2	5.8	47	2.3	6.4	18	74
Case 3	3.5	52	3.0	6.1	18	77
Case 4	5.4	71	8.1	6.8	21	103
Case 5	6.2	109	8.1	9.3	23	144
Case 6	7.5	214	14.5	18.4	52	285
Case 7	7.3	234	19.2	21.0	51	306

When the corpuscles were analyzed for these various components, by washing the cells after centrifuging and decanting the super-

[1] Berglund, H. (Professor of Medicine, University of Minnesota), see page 809.

natant plasma and then hemolyzing them, the following data were obtained:

	NH_2.	Urea.	Creatinin.	Uric acid.	Undetermined N.	N. P. N.	Number of mls. of cells per 100 mls. of whole blood
Case 1.....	5.9	13	1.8	3.7	18	39	45
Case 2.....	7.2	52	1.5	1.6	...	51	50
Case 3.....	7.4	41	3.0	0.9	31	81	27
Case 4.....	7.2	26	6.5	...	86	122	23
Case 5.....	11.8	38	4.4	...	93	144	25
Case 6.....	9.7	113	10.5	6.1	109	238	39
Case 7.....	7.3	138	11.7	...	43	193	42

Methods for Determining the Non-protid Nitrogen of Blood.—*Direct Nesslerization Method of Folin.*[1]—Preparation of the non-protid solution. In order to obtain the protid-free solution for analysis, the following procedure is recommended by Folin: Principle: Blood is collected in tubes provided with an oxalate as anticoagulant.[2] The best proportion of oxalate to blood is 20 mgs. of potassium[3] oxalate for the usual amount of blood drawn for analysis, namely, 10 mls.[4] Then the blood is hemolyzed if the determination is to be made on whole blood; this is done by adding a hypotonic solution (water), which causes the osmotic pressure within the corpuscles to become greater than the tension of the cell walls, with the result that the corpuscles become disrupted, freeing to the liquid any nitrogenous substances that the cell may contain. The protid (stroma of the corpuscles, plasma albumin, and globulin, etc.) is then coagulated by means of tungstic acid (sodium tungstate in the presence of sulphuric acid) and the solution is then filtered. The determinations are made upon the clear, protid-free filtrate.

Procedure: Pipette 10 mls. of the blood from the test-tube in which it was collected to a small Erlenmeyer flask. Add 70 mls. of

[1] The methods of Folin described in these pages were originally published in the Jour. Biol. Chem., vol. 38, 1919, and later. The work was accomplished in conjunction with H. Wu (Pekin-Union Medical School, Pekin, China). Some of the methods have been modified by Folin and Wu and by others in minor details. For frequent additions, alterations, and supplements the journals must be referred to, as well as Folin's Laboratory Manual, 4th ed., 1925.

[2] The oxalate forms calcium oxalate, which is insoluble. When calcium is not present in coagulating fluids, like blood and milk, the protid, although it may be in a changed condition, only becomes precipitated when it is converted into an insoluble salt. Calcium performs this function. By removing Ca the blood does not coagulate.

[3] Folin advocates the use of lithium oxalate, because potassium salts produce undesirable precipitates, especially with the reagent used in the determination of uric acid.

[4] Excessive amounts of oxalate interfere with the proper filtering of the blood after adding the coagulating reagent and the uric acid determination is less dependable.

See page 67.

distilled water from a cylinder. Mix thoroughly. Then add with a pipette 10 mls. of the reagent[1] sodium tungstate. Nearly fill your 15-ml. pipette with the two-thirds normal sulphuric acid and add 10 mls. slowly to the contents of the flask while gently agitating the contents. Stopper the flask and continue the mixing. The solution is proper for filtering if only a slight amount of foam has formed and if the color of the solution becomes chocolate brown.[2] Place a funnel that has a capacity of about 120 mls.[3] in your rack. Provide the funnel with a dry paper, wetted after it is in place, with a few mls. of the solution. Then pour the whole of the well-mixed solution upon the paper. The filtrate must be crystal clear. If it is not, pour the filtrate upon the residue in the funnel and rinse the receiving vessel; or better, use another vessel which is dry. When enough filtrate has passed proceed to the determination. If the filtrate is not to be used until a following period, add a small amount of sodium fluorid as a bactericidal agent.

The Determination of the Non-protid Nitrogen.—Principle: The analysis is made by a modified Kjeldahl process. Procedure: Pipette 5 mls. of the filtrate obtained by the above method into a 200 × 20 mm. Pyrex test-tube. The tube should be dry. If it has been recently used, wash with water, drain, and then rinse with alcohol. Add 1 ml. of the special acid mixture and a "bumping-piece," a quartz pebble or a piece of broken Pyrex tube. Fasten the test-tube on a stand 15 mms. above a microburner and heat until white fumes appear. Then cover the mouth of the tube with an inverted porcelain crucible cover and continue heating with a reduced flame.[4] Note the appearance of the charring in the boiling liquid in the bottom of the tube. Continue the almost imperceptible boiling until the brownish-black color gives place to a yellowish-green, and this, in turn, to a clear green. Usually about two minutes elapse between the appearance of the white fumes and the completion of oxidation. Next permit the tube to cool for about one minute and a half. Add slowly[5]

[1] Appendix.

[2] Failure of the coagulum to answer these two requirements is ordinarily due to excessive amounts of tungstate or oxalate. In such cases carefully add, with gentle shaking, 10 per cent. H_2SO_4 solution, drop by drop, until the foam disappears and the color assumes the proper shade. If this does not occur promptly, the preparation should be rejected and a new one made.

[3] An ordinary 60-degree glass funnel having a long stem and capacity of about 100 mls. should be used.

[4] The flame should not be more than 1 cm. in height.

[5] If cold water is added quickly to the hot acid, some of the contents of the tube may be lost, owing to the sputtering when the two liquids are mixed.

from your wash-bottle water to make the total volume about 25 mls. Transfer the contents of the tube to a 50-ml. volumetric cylinder;[1] make up the volume to 35 mls. with distilled water and to 50 mls. with the special Nessler solution. Stopper the cylinder, or tube, and invert several times in order to mix the contents. The mixed solution must be clear. If it become murky, mix well and transfer some to a 15-ml. centrifuge tube and centrifuge.[2] Use the supernatant liquid to half fill the cup. To the other cup add a similar amount of the standard solution made by the following method: Pipette three 1-ml. portions of the special ammonium sulphate solution into a 100-ml. volumetric flask. Add 2 mls. of the acid mixture used in the oxidation of the unknown and dilute with water to about 60 mls. Then add 30 mls. of the Nessler solution at the same time that you add Nessler solution to the unknown. Make up to 100 mls. with distilled water. Stopper the flask, invert, mix, and transfer enough to the second cup of the colorimeter to make the cup half full. Compare the color of the two solutions in the colorimeter, setting the standard (left) at 20 mls.

Calculation:

$$\frac{R_s\ (20)}{R_u\ (n)} = \frac{C_u\ (x)}{C_s\ (0.3\ \text{mg.})}.^{3}$$

Then:

$$nx = 6$$

and

$$x = \frac{6}{n}\ \text{mgs. of nitrogen in 10 mls. of 1:10 solution.}^{4}$$

Or:

$$x = \frac{60}{n}\ \text{mgs. in 10 mls. whole blood.}$$

Or:

$$x = \frac{600}{n}\ \text{mgs. for 100 mls. of whole blood,}^{5}\ \text{that is, per cent.}$$

[1] If a special Folin digestion tube (Fig. 239) is available, the whole procedure may be carried on in it. The tube is marked at 35 mls.

[2] If the precipitate is colored indicating loss of Nessler compound the experiment must be repeated, as only murkiness due to silicate can be removed by centrifuging.

[3] Three-tenths of a milligram, since the standard solution of ammonium sulphate is so made that each 10 mls. contain 1 mg. of nitrogen and each ml., 0.1 mg. nitrogen. Three mls. of this solution (containing 0.3 mg. of nitrogen) are diluted to 100 mls.; this solution is used to match the unknown.

[4] Five mls. of whole blood were made up to 50 mls. during the process of precipitation of the protids. Five mls. of the filtrate were taken for the determination of N. P. N. and diluted to 50 mls; as the standard is diluted to 100 we calculate on the basis of 10 mls. of filtrate diluted to 100 mls.

[5] That is, there are ten 10 ml. amounts in 100 mls. of blood; $10 \times 60 = 600$.

Other Procedures for N. P. N.—The method given above may be modified by titrating, as in the *standard Kjeldahl method:*[1] After obtaining the clear solution at the end of boiling, proceed as follows: Cool, dilute with about 7 mls. of water, and insert into the mouth of the tube a stopper carrying a long tube provided with a piece of rubber tubing and a clamp (Fig. 50[2]) and a short delivery tube for attaching to a small condenser. Three mls. of 50 per cent. NaOH solution have been sucked up into the long tube and held by the clamp. Place 2 mls. of decinormal hydrochloric acid in a 100-ml. volumetric flask and see that the delivery tube from the condenser dips beneath the surface of the acid solution. When all attachments have been made open the clamp and permit alkali to neutralize the acid in the test-tube. Now heat the bottom of the tube freely in a flame to cause vigorous boiling. Discontinue when a residue of salts appears in the bottom of the tube and "bumping" occurs. Disconnect the condenser, wash off the drops of HCl from the delivery tube dipping into the volumetric flask, and titrate the contents against 0.1 normal NaOH solution as in the Kjeldahl method.[3]

If one desires to obtain all amino- and peptid-nitrogen, the trichloracetic method of precipitation of the protids recommended by Greenwald[4] may be used with the above procedure of Benedict, or with that suggested by Myers.[5]

Non-protid Nitrogen by Myers' Method (Somewhat Modified).—Pipette into a 200 × 20 mm. Pyrex test-tube 3 mls. of whole blood, obtained from a vein and prevented from coagulating by the method given for the Folin procedure. Add 15 mls. of distilled water and enough 5 per cent. trichloracetic acid to make a total volume of 30 mls.; this gives a 1:10 solution (note for the purpose of calculation). Stopper the tube and mix. Let stand half an hour. Filter through a dry paper moistened with some of the liquid. Rinse out the test-tube and use it to receive the filtrate. Pipette 10 mls. of the filtrate into a thin-walled test-tube of such a size that it can be slipped into a 100-ml. volumetric cylinder. The cylinder carries a short tube that

[1] Page 290. The procedure given here is recommended by Benedict. See Bock, J. C. (Marquette University, Milwaukee, Wisconsin), and Benedict, S. R., Jour. Biol. Chem., vol. 20, p. 47, 1915.

[2] For the present purpose the apparatus, as shown in the figure, is inverted.

[3] The use of a volumetric flask for receiving the distillate is unnecessary, if one does not care to use the Benedict procedure for Nesslerization as in the Folin method given on page 814. Benedict has recommended the above procedure as giving more accurate results.

[4] Greenwald, I., Jour. Biol. Chem., vol. 21, p. 61, 1915.

[5] Myers, page 806.

dips just beneath the two-hole rubber stopper fitting the cylinder and a second, longer one which passes through the other hole in the stopper to the bottom of the test-tube within the cylinder. Oxidize the substances in the filtrate in the test-tube according to the principle of the Kjeldahl method: Add to the test-tube about 0.2 g. potassium sulphate crystals, 2 drops of 10 per cent. $CuSO_4$ solution, and about 0.5 ml. of concentrated H_2SO_4. Boil the mixture over a microburner as in the Folin method. Continue boiling two minutes

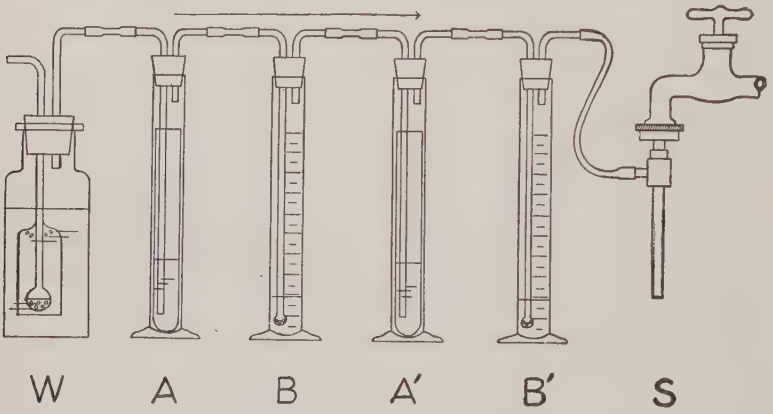


Fig. 241.—Aëration apparatus for estimation of urea and ammonia: W, Bottle containing dilute sulphuric acid to remove ammonia from the air which passes through the apparatus, A, A′, cylinders in which the test fluid is placed; B, B′, cylinders with decinormal acid to receive the ammonia; S, suction. The arrow flies with the air current. Method of Victor Myers, see page 24. (From Todd, Clinical Diagnosis by Laboratory Methods.)

after the solution has passed through the charring stage and has become clear green. Let the tube stand for the purpose of cooling for about two minutes and dilute the contents with 6 mls. of distilled water. Lower the test-tube into the second of a series of two volumetric cylinders (Fig. 241), and in the first one place 15 mls. of distilled water and 2 mls. of 0.1 normal HCl solution. Affix to the cylinder containing the unknown solution a wash-bottle[1] containing not less than 150 to 200 mls. of 10 per cent. sulphuric acid in order to remove the ammonia existing in the air that passes through the system. The wash-bottle should contain an indicator, like Congo-red, to show when the acid becomes neutralized by the ammonia

[1] Benedict has shown that a large volume of acid must be used in any aspiration method in order to catch all the ammonia introduced from the air which the pump aspirates through the system of cylinders. See the reference to Bock and Benedict, page 817.

from the air. When all is connected up, remove the rubber tubing from the longer tube in the second cylinder and introduce by means of a pipette 3 mls. of 50 per cent. NaOH solution to neutralize the acid in the test-tube. Replace the tubing and start a current of air, at first slowly and then as rapidly as possible, without any risk of drawing one solution into another. Continue aspirating for half an hour. While the current of air is still passing through the apparatus, carefully disconnect the tubing between the cylinders, thus avoiding any back-flow.

Titration or Nesslerization May Be Used.—The latter process is as follows: Pipette into a 100-ml. volumetric flask 5 mls. of the standard ammonium sulphate solution, equivalent to introducing 1 mg. of nitrogen. Dilute to about 60 mls. with distilled water. Add 20 mls. of the special, diluted Bock-Benedict Nessler solution (Appendix). Now turn your attention to the Nesslerization of the unknown in the test tube: Add 8 mls. of the diluted Nessler solution to the contents of the test-tube and make up the volume to 25 mls., transferring the liquid to a cylinder if necessary. Dilute the standard solution to the mark, stopper, mix, and transfer some of the liquid to the standard (left) cup of the colorimeter.[1] Transfer, also, some of the unknown solution to the right-hand cup (or to the cylindrical one, C, of the Bock-Benedict colorimeter), and compare the colors.

Calculation:[2]

$$\frac{R_s}{R_u} = \frac{D_u}{100} \times 1 \times 100 \text{ mg. gives the mgs.N. P N.. in 100 mls. of blood, where}$$

R_s is the reading of the standard.
R_u is that of the unknown.
D_u is the dilution of the unknown.
100 is the dilution of the standard.
1 is the number of mgs. in the standard.
100 is necessary for the conversion to mgs. per ml.

Gasometric Micro-Kjeldahl Method of Van Slyke.[3]—Principle: The principle is identical with that of the macro-Kjeldahl method in which Folin's use of sulphuric-phosphoric acid mixture but without cupric sulphate, is repeated. Then, in place of titration or colorimetric procedure, a determination of ammonia nitrogen is made by means of the gasometric apparatus, nitrogen being freed as molecular nitrogen by the hypobromite method (page 704). Reagents:

[1] If the Bock-Benedict colorimeter is used (Fig. 249), the standard goes into the cup provided for that purpose. See Bock, J. C., and Benedict, S. R., Jour. Biol. Chem., vol. 35, p. 227, 1918. The standard cup is situated between the mirrors.

[2] For the Duboscq type of colorimeter.

[3] The author is under obligation to Dr. Van Slyke for the details of the method.

Into 100 mls. of phosphoric acid (85 per cent. syrupy "ortho"), pour slowly 300 mls. of concentrated sulphuric acid (sp. gr. 1.84). Label this "acid mixture for Van Slyke method."

Potassium persulphate, $K_2S_2O_8$, freed from NH_3 as follows: Dilute one part by weight with 10 parts by weight of water warmed to about 60° C. Using litmus paper as indicator, make strongly alkaline by means of 40 per cent. NaOH solution. Distil, preferably under vacuum of less than 40 mms. of mercury until about a third has been distilled over, then add approximately this amount of the warm water, enough NaOH to restore the alkalinity of the solution and distil farther. The process may have to be repeated in order to obtain ammonia-free persulphate. Then pour the solution into a beaker and leave in the ice-box over night, to crystallize. The crystals are washed on a Buchner funnel with ice water, the residue sucked as dry as possible and then placed in a vacuum desiccator over sulphuric acid.[1]

Sodium hypobromite solution: To 50 mls. of 40 per cent. NaOH solution in a 250 ml. flask, add, in a hood, 1 ml. of liquid bromin. Stir until the bromin is completely dissolved. In making this addition, swirl the contents of the flask about the walls of the flask in order to aid the escape of oxygen. This solution should be made fresh each half-day of laboratory work.

Procedure: Into a 200 × 20 mm. Pyrex digestion flask pipette, accurately such an amount of the unknown nitrogen solution as will contain about 1 mg. nitrogen.[2] Add 1 ml. of the acid mixture and a quartz pebble. Boil until the characteristic white fumes appear, cool, add about 1 g. of persulphate and 1 ml. of water. Continue heating[3] until the solution is entirely clear. Cool, add 3 mls. of water and one drop of alizarin indicator. From a burette add NaOH 40 per cent., drop by drop (keeping cool); the heat generated will melt the slag at the bottom of the tube. Continue until the alizarin indicates an alkaline reaction. From a Mohr pipette, add, now, 10 per cent. H_2SO_4 to slight acidity, in order to prevent loss of ammonia. Transfer, quantitatively, the solution to the burette of the gasometer and measure the volume. By proper manipulation of the cocks, draw the solution into the mixing chamber. By means of a pipette, rinse the test-tube with enough water to bring

[1] Merck and Co., New York, N. Y., supply a product free from ammonia. This concern has become a part of Powers Weightman and Rosengarten, Philadelphia.

[2] For urine, dilute the specimen 1:10 and take 1 ml. For blood filtrate, take 10 mls.

[3] Reduce the flame of the microburner from this point.

the total volume up to 10 or 11 mls. The determination: Seal the cock of the gas chamber with a drop of mercury, evacuate the chamber and shake three minutes; eject the air. Repeat once. Now take the flask holding the hypobromite solution in one hand and swirl it about in order to dislodge any accumulated oxygen; pipette 1.5 mls. of the solution to the small burette above cock b and permit 1 ml. of it to pass into the chamber. Pipette away the remaining 0.5 ml., seal the burette with a drop of Hg, evacuate and shake for 3 minutes. Note the temperature. Reduce the gas volume to 2.0 mls. and read the manometer as p_1. Eject the gas from the chamber, lower the meniscus to the 2.0 ml. mark and read p_2 on the manometer. Calculation:

Mgs. nitrogen in sample taken
$$= (p_1 - p_2 - c) \text{ times } f$$

in which c is the $(p_1 - p_2)$ of the blank and f the mgs. nitrogen corresponding to 1 mm. pressure at the temperature of the determination. This factor is found from the following table:

Temperature.	*Factor.*
15° C.	0.00 32 50
16	41
17	27
18	15
19	01
20	0.00 31 92
21	80
22	68
23	56
24	45
25	0.00 31 36
26	24
27	16
28	04
29	.00 30 92
30	0.00 30 80
31	71
32	60
33	50
34	40

Table giving the factors by which mm. pressure at 2.0 ml. volume must be multiplied in order to obtain mgs. nitrogen. The correction factor 1.04, for the fact that hypobromite gives only 96 per cent. of the theoretical amount of nitrogen, is included in the factors in this table.

UREA

Urea nitrogen represents about one-half of the non-protid nitrogen of normal blood. The proportion varies in disease, as may be seen by consulting pages 813 and 814. It varies with the intake of nitrogen in the foods. The average normal urea concentration in human blood is 12 mgs. per 100 mls. of whole blood 1.5 to two hours after a meal. On a diet high in protid the content of urea nitrogen is high. It is low during fasting for short periods, but rises after prolonged starvation. Folin gives the normal ranges of blood urea nitrogen as 8 to 15 mgs., the latter figure being found only on exceptionally high protid diets. The change from a low to a higher blood urea nitrogen level, or vice versa, owing to changes in diet, is not immediate. Days may elapse before the change in the blood becomes evident. The following table shows the effect upon urea nitrogen of introducing urea into the intestine (after Folin):

	Mgs.
Urea nitrogen absorbed from the intestine	1436
Portal blood urea nitrogen before injection	23
Carotid blood urea nitrogen before injection	23
Portal blood urea nitrogen after injection	154
Carotid blood urea nitrogen after injection	92

The distribution of urea nitrogen in blood throughout the body during rest periods is uniform, but two or three minutes after meals there is a rise, at first in the portal system, which carries the absorbed urea from the intestines to the liver, and thence through the body. Within half an hour the urea nitrogen in carotid blood increases to not more than eleven times normal.

In *pathological states* urea shows prompt response to any renal lesion. From the table on page 824 it may be seen that the increase in urea nitrogen is proportionately greater than in any other constituent of the N. P. N. It is for this reason that attempts have been made to make a quantitative relation between blood and urinary urea and certain factors that vary with individuals. Ambard[1] suggested a formula involving the ratio: Blood urea to urine urea, modified by the weight of the subject. The formula is based on the assumption that the rate of excretion of urea varies as the square of blood urea and also that excretion of urea depends upon the number of grams per unit volume, regardless of volume of urine. Equation of Ambard is:

[1] Ambard, L. (University of Paris). See Physiologie Normale et Pathologique des Reins, Paris, Masson et C^ie^, 2d ed., 1920.

$$\text{Coefficient} = \frac{\text{Gs. urea per 1000 mls. blood}}{\sqrt{\text{Gs. urea per 24 hrs.} \times \frac{70}{\text{Body weight}} \times \sqrt{\frac{\text{Gs. urea per 1000 mls. urine}}{25}}}},$$

70 being average normal body weight and 25 average normal twenty-four hour excretion of urea (dry).

The normal coefficient is 0.08. McLean[1] used this coefficient as 100 per cent. as a standard expressing the degree of urea retention caused by inefficiency of the kidney. Van Slyke and his colleagues found that the rate of excretion varies in a linear way, that is, as the first power of blood-urea and as the square root of the volume of urine:[2]

$$C = \frac{\text{Grams of urea excreted per twenty-four hours}}{\text{Blood urea per liter} \sqrt{\text{Urine volume per twenty-four hours} \times \text{kilos body weight}}}.$$

The coefficient normally is $C = 7.5 \pm 3$. The coefficient becomes less during retention.

For ready calculation the firm of Keuffel and Esser provides a special Mannheim type slide-rule (Fig. 242) with gauge-points.

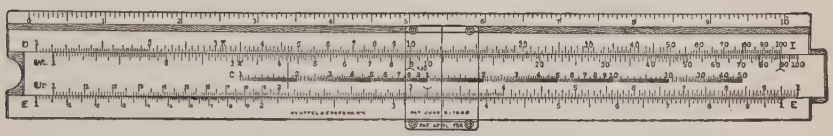


Fig. 242.—The McLean calculating rule for urea concentration. By simple manipulation the McLean Index is read. (Kindness of the Keuffel and Esser Co., Hoboken, N. J., who supply both rule and directions for its use.) The rule is adapted to many biochemical calculations.

Concerning these attempts at quantitative expressions, Folin says: "The idea of the existence of such a constant certainly breaks down when it is extended so as to account for the rate of excretion of all waste products." Myers says it "afford a basis for detecting the abnormalities in urea excretion, but not for interpreting the significance of such abnormalities, since it was largely physiological in its nature."

The following Table gives the *characteristic variations of blood urea nitrogen in different pathological states:*

Normal or low: As in the case of N. P. N. Especially in nephrosis, 13 mgs. per cent.

High: Also as in the case of N. P. N. In addition: Urea represents the second step of Myers' "staircase,"[3] by which is meant that renal lesions first affect excretion of uric acid, then of urea, and finally of creatinin (Table, page 824):

[1] McLean, F. C., and Selling, L.: Jour. Biol. Chem., vol. 19, p. 31, 1914.

[2] Up to a certain limit. In diabetes, in which there is an excretion of several liters of urine, the results become irregular.

[3] See Chase, A. F., and Myers, V. C., Jour. Amer. Med. Assoc., vol. 67, p. 929, 1916.

TABLE SHOWING THE "STAIRCASE" OF MYERS

	Mgs. per 100 mls. blood: Uric acid.	Urea N.	Creatinin.
Tuberculosis, pulmonary	6.5	16	2.7
Pericarditis	5.6	13	2.1
Interstitial nephritis	5.5	12	2.5
Diffuse nephritis	9.6	19	2.4
Early interstitial nephritis	9.5	25	2.5
Early interstitial nephritis	6.6	24	3.3
Early interstitial nephritis	8.7	20	3.6
Early interstitial nephritis	6.3	31	2.0
Chronic interstitial nephritis	8.0	80	4.8
Chronic diffuse nephritis	8.3	72	3.2
Chronic diffuse nephritis	9.5	44	3.5
Chronic interstitial nephritis (fatal)	22.4	236	16.7
Chronic interstitial nephritis (fatal)	15.0	240	20.5
Chronic interstitial nephritis (fatal)	14.3	263	22.2
Chronic interstitial nephritis (fatal)	8.7	144	11.0

Methods for the Quantitative Determination of Blood-urea Nitrogen.—Urease is the basis of the modern methods for the determination of urea in blood. Two possibilities in its use exist, namely, (1) Determine the ammonia from the $(NH_4)_2CO_3$ produced under action of the enzyme, titrating or Nesslerizing in the analysis. (2) Determine the CO_2 derived from the ammonium carbonate. Both methods are in use, the former method is utilized by Folin and others (see below) and the latter by Van Slyke in his new manometric, gasometric methods of analysis[1].

Method of Folin.—Principle: The blood protids are precipitated by the method given under non-protid nitrogen. A measured portion of the filtrate is then treated with urease and the ammonia formed in the reaction is distilled into standard acid as in the Kjeldahl method. After Nesslerization of the distillate color comparison is made, as in the N. P. N. method.

Procedure: Using a clean[2] 200 × 20 mm. Pyrex test-tube, place 5 mls. of the filtrate obtained by the blood-precipitation method, given under non-protid nitrogen (page 815), in it, add 1 ml. of the special urease solution[2] and 2 drops of the special buffer mixture.[3] Leave the tube in a water-bath at not over 55° C. for five minutes, or, if the room temperature is above 20° C., the tube may be left for not less than fifteen minutes in the test-tube rack. Then proceed to the distillation:

[1] Van Slyke, D. D. Determination of urea by gasometric measurement of the carbon dioxide formed by the action of urease. Jour. Biol. Chem., vol. 73, page 695, 1927.

[2] If the tube has been used for Nesslerizations, it must be cleaned with concentrated HNO_3, then thoroughly washed with tap-water, and finally with distilled water. Nessler's solution, owing to the mercury it contains, is a poison to the enzyme, urease.

[3] Appendix.

Introduce into the tube a cork through which a long piece of glass tubing, bent into a wide ∩ is pushed (Fig. 243) to serve as delivery tube to the second test-tube, prepared as follows: Place 2 mls. of 0.05 normal HCl solution in another test-tube[1] and introduce into it the delivery end of the ∩-tube, taking care that the apertures of the tube (Fig. 243) dip below the surface of the acid. To the first test-tube, add a boiling-piece (quartz pebble), 1 drop of foam-preventing agent (paraffin oil; albolene) and 2 mls. of a saturated

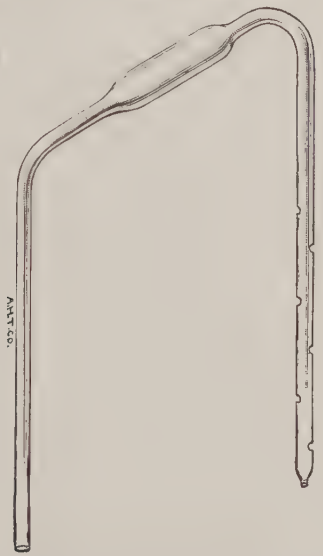


Fig. 243.—Watson and White modification of the Folin-Wu connecting tube for urea determinations. The apparatus is designed to avoid frothing and back-suction during distillation of the ammonia. See Watson, T., and White, H. L. (Univ. Southern Cal., Los Angeles), Jour. Biol. Chem., vol. 45, p. 465, 1921.

solution of borax; then insert the stoppers.[2] Apply heat gently, but continuously,[3] to the bottom of the distilling tube for four minutes. Then slightly withdraw the receiving tube, and leave it in this posi-

[1] One may obtain on the market special Folin urea receiving tubes graduated at 25 mls. The tube of Nash (Fig. 247) may also be used. Or an ordinary tube may be marked with glass-marking pencil to indicate this volume.

[2] If desired the delivery tube may be provided with a second cork fitted into the second test-tube containing the acid. *A vertical slit must be cut in the sides of the cork* to permit egress of the air displaced by the distillate.

[3] The beginner will experience much difficulty in that the small variations in the flame used to cause distillation will permit back-suction of the contents of the receiving tube. In order to avoid this difficulty it is best to provide classes of students with the apparatus shown in Fig. 243; or one may use ungraduated 5-ml. pipettes, the bulb serving as a safety device. A mica chimney for the microburner reduces the variations in the flame (Fig. 248).

tion while distillation progresses for another minute. With a stream of water from your wash-bottle wash the lower end of the delivery tube. Cool the contents of the receiving test-tube under the cold water-tap. Add distilled water to make a volume of about 20 mls.

Make the standard: Pipette 3 mls. of standard ammonium sulphate solution into a 100-ml. volumetric flask, dilute to about 75 mls., and Nesslerize by adding 10 mls. of Nessler's solution. At the same time add 2.5 mls. of Nessler's solution to the receiving test-tube; dilute the solution in the flask and tube to their respective marks (100 mls. and 25 mls.), stopper, mix, and read in a colorimeter.

Calculation: The calculation is like that given for N. P. N.[1] (page 816); or the reading of the standard may be placed at 20 mms., that number divided by the reading of the unknown and the result multiplied by 15. The amount thus obtained is the number of milligrams of urea nitrogen in 100 mls. of whole blood.

Method of Myers.—Principle: Urease is used to remove the ammonia from the urea as in the method of Folin. Then the ammonia is aspirated by means of an air current into standard acid, using the apparatus required for Myers' method for N. P. N. (Fig. 241). Whole blood is usually used in place of blood filtrate, although the latter may be used.

Procedure: Pipette 2 mls. of oxalated whole blood into a thin-walled test-tube which can be fitted into a volumetric cylinder. Add 1 ml. of the special urease solution and 2 drops of the special buffer mixture used in the Folin method. Leave the tube in a beaker of water at not over 55° C. for fifteen minutes or longer. Add 4 drops of caprylic alcohol to prevent foaming and prepare the receiving cylinder by pipetting exactly 2 mls. of decinormal hydrochloric acid into it and also adding 4 drops of caprylic alcohol. Have all connections ready and then to the blood tube add 5 mls. of 50 per cent. sodium carbonate solution. Continue aërating for about half an hour. The rest of the procedure is like that for N. P. N. (page 815). These two procedures may be conducted simultaneously.

AMMONIA IN THE BLOOD

Nash and Benedict[2] have demonstrated that ammonia production is controlled by the kidney. The process is concerned with the regulation of the alkalinity of the organism, by protecting it against

[1] The principle is the same, but Nesslerization is made for N. P. N. on 50 mls. and for urea at half that amount.

[2] Nash, T. P., and Benedict, S. R. (Cornell Medical, New York, N. Y.), Jour. Biol. Chem., vol. 48, p. 463, 1921.

depletion of the alkali reserve. Interference with the ammonia production by the kidney leads to acidosis, because the acid radicles are not neutralized by the ammonia and hence they accumulate in the blood, thus depleting the alkali reserve (bicarbonate, fixed bases and protid). The amount of ammonia in human blood is small. Folin found a fraction of a milligram per 100 mls. of blood; Denis the same. Other observers[1] find 0.35 mg. ammonia in the blood of the dog. Whatever the average amount is, it has no significance clinically. The determination is one of great difficulty. A satisfactory method has been published by Nash and Benedict[2] as a modification of the Folin procedure.

URIC ACID IN BLOOD

Uric acid occurs in human blood in several forms. It probably occurs as free uric acid in the form of β-urate (page 720), as a β-urate with sodium and in combination with other substances. Thus Benedict and his coworkers have found uric acid combined with a pentose, ribose.[3]

The determination of uric acid involves uric acid as such, and not uric acid nitrogen. The average normal amount of uric acid in human blood is 3.4 mgs. per 100 mls. of whole blood.[4] The distribution is uniform for both arterial and venous blood; this is shown for the dog's blood as follows:

	Uric acid per 100 mls. plasma. Arterial, mgs.	Venous, mgs.
Five minutes after injecting 200 mgs. uric acid.....	66.8	65.6
Three minutes after injecting 100 mgs. uric acid.....	28.2	28.4
Three and a half minutes after 100 mgs. are injected..	41.2	44.4

The distribution of uric acid between serum and corpuscles is shown by the following table from Benedict and co-worker:[5]

Where the whole blood uric acid is	The serum uric acid is	The corpuscular uric acid is
3.4 mgs. per cent.	3.1 mgs. per cent.	3.4 mgs. per cent.
2.8 " "	3.0 " "	1.8 " "
3.6 " "	3.4 " "	3.7 " "
2.6 " "	3.0 " "	1.8 " "
6.0 " "	6.4 " "	2.3 " "

[1] Matthews, S. A., and Miller, E. M. (Rush Medical College; Matthews now of Loyola University, Chicago, Ill.), Jour. Biol. Chem., vol. 15, p. 87, 1913.

[2] Page 826.

[3] Compare a purin-glucid combination, the free mononucleotid (page 339) in which, in place of a tri-oxypurin, uric acid, a mon-oxypurin, hypoxanthin is united with a pentose, ribose.

[4] In the laboratories of Jefferson Hospital it is rare to find a uric acid content lower than 3 mgs. per cent. in normal cases.

[5] Theis, R., and Benedict, S. R., Jour. Lab. and Clin. Med., vol. 6, p. 1, 1921.

The uric acid is destroyed in the blood. This is shown in the following table from Folin[1] (dog):

	Uric acid per 100 mls. plasma.
100 mgs. of uric acid injected per kilo body weight in four minutes. Immediately after injection intravenously, blood plasma had	72.8 mgs.
3 minutes after injection	30.6 "
4 minutes after injection	25.4 "
16 minutes after injection	12.1 "
17 minutes after injection	10.8 "

Such destruction of uric acid in the blood does not occur if the blood is removed from circulation. Tissues take from the blood certain amounts of uric acid. Normally, the kidneys are especially able to absorb it. In gout, the power of the kidney to absorb uric acid becomes modified and blood of gouty persons may contain high quantities of uric acid.

Subject K-z of Folin, May 11, 10.7 mgs. per cent. uric acid (plasma).
Subject K-z of Folin, May 22, 8 mgs. per cent. uric acid (plasma).
Subject B-th of Folin (attack), 9.2 mgs. per cent. uric acid (plasma).

The uric acid of circulating blood is that which (1) has not been destroyed in the blood, and (2) cannot be absorbed by the kidney.

The following table gives the *variations in uric acid concentration in blood under physiological and pathological conditions* (whole blood):

Physiological variations:

Low concentrations:

Of unknown nature	1 mg. per 100 mls.
Due to low protid diet	1 to 3 mgs. per 100 mls.
Due to drugs (analgesics)	Less than 3 mgs. per 100 mls.
High protid, purin free	3 mgs. per 100 mls.[2]

High concentrations:

Due to age (first five days postnatal)	Above 3 mgs.[3]
High purin diet	3 to 5 mgs. per cent.
Causes unknown	3.8 mgs.

[1] Folin, O., Berglund, M., and Derick, C., Jour. Biol. Chem., vol. 60, p. 361, 1924.

[2] From Folin, Berglund, and Derick (see page 722), page 437. The subject had been on a high-protid purin-free diet for ten days. The total urinary nitrogen was over 30 per cent. above the average normal for man, which indicates the character of the diet.

[3] Kingsbury, F. B. (page 23), and Sedgwick, J. P. (pediatrist, Minnesota, deceased). See Jour. Biol. Chem., vol. 31, p. 261, 1917.

Pathological variations:

Low uric acid concentrations (or normal):

Recovery from acute nephritis	2.5 mgs. per cent.
Neurasthenia	1.7 mgs.
Pregnancy	2.3 mgs. (normal)
Arteriosclerosis (may be higher)	1.9 mgs.
Cancer (average of 15 dets., Theis)	3.0 mgs. (normal)
Nephrosis	Normal to 5 mgs.

High uric acid concentration:

Incipient interstitial nephritis[1]	4.0 mgs. per cent.
Chronic interstitial nephritis (usual)	6 to 15 mgs. per cent.
Chronic interstitial nephritis (rare)	15 to 27 mgs. per cent.[2]
Gout during attack	9.2 mgs. per cent.
Gout between attacks	7.2 " "
Prostate obstruction	3.9 " "
Bichlorid poisoning, up to	20.0 " "
Toxemias of pregnancy up to	12.0 " "
Leukemia, fatal	10.0 " "
Lead poisoning	7.0 " "
Infections (pneumonia, before crisis)	6.0 " "

[1] May be higher.

[2] This is a case cited by Myers (Myers, V. C., and Fine, M. S., Jour. Biol. Chem., vol. 20, p. 391, 1915.

Methods for Determining Blood Uric Acid.—The exact methods for determining small amounts of uric acid in blood resulted from the suggestion of Folin in 1912[1] that the blue color which appears when uric acid in alkaline solution is treated with phosphotungstic acid solutions can be utilized for quantitative purposes. Other substances[2] than uric acid give this color under similar conditions, and the chief modifications that have been made in the *original Folin method* concern procedures which make the reagent specific for uric acid enhance the color and simplify the determination. Benedict laid the foundation for the most rapid method when he discovered that sodium cyanid frees uric acid from certain compounds, and that it may be used to replace some of the phosphoric acid used in the reagent. The older methods used an alkali (sodium carbonate), but in the latest modification of Benedict cyanid replaces the alkali. In the making of standard solutions for the colorimetric comparisons

[1] Folin, O., and Denis, W., Jour. Biol. Chem., vol. 13, p. 469, 1912. Later papers in the same journal matured the work. The color reaction was considered in the two following papers: Folin, O., and Macallum, A. B., Jour. Biol. Chem., vol. 11, p. 265, 1912; same journal, vol. 12, p. 239, 1912 (Folin and Denis).

[2] Like Millon's reagent; phosphotungstic acid produces color in substances having an hydroxyl radicle in the para position on the aromatic nucleus (page 241).

Folin suggested the practical application of formaldehyde to form formaldehyde-uric acid compounds, which, when their solutions were diluted with water, liberated uric acid. These compounds resist alterations of the uric acid portion, and yet when the acid is ready for use, it is easily obtained from the formaldehyde-uric acid compound.

Method of Benedict.[1]—Principle: Protids are precipitated from whole blood according to the method of Folin (page 814) and an aliquot portion of the filtrate is treated with cyanid solution to render it free in solution and to afford alkali necessary for the production of color. Then arsenic-tungstate solution is added as arsenic intensifies the color. The preparation is heated to accentuate the characteristic color still more, and after cooling the tubes the colors are compared at once to avoid development of turbidity. The standard is made from a solution of uric acid in a special buffer mixture which just holds the acid in solution,[2] yet prevents oxidation and consequent lowering of the concentration of uric acid.

Procedure: Pipette 5 mls. of the filtrate into a 200 × 20 mm. test-tube and add 5 mls. of distilled water. Make the standard: Pipette 5 mls. of standard solution[3] into a second tube and make the volume up to about 10 mls. of solution. To both tubes add 4 mls. of 5 per cent. sodium cyanid solution[4] (Caution!) from a burette and also 1 ml. of the special arsenic phosphoric solution. Stopper the tubes, immediately invert them to mix the contents; then place at once in the boiling water-bath. Leave three minutes and then transfer to a large beaker of cold water.

[1] Benedict, S. R., Jour. Biol. Chem., vol. 51, p. 187, 1922. The reagents are given in the Appendix. Concerning thiazin, which gives a color reaction similar to that given by uric acid, see page 832.

[2] The method is described in Benedict, S. R., and Hitchcock, E. H., Jour. Biol. Chem., vol. 20, p. 619, 1915.

[3] The dilution is made whenever a determination is to be made, since the solutions keep better in concentrated form; a weaker and a stronger standard are ordinarily used. For the weaker, proceed as follows: Pipette 5 mls. of the Benedict-Hitchcock solution into a 500-ml. volumetric flask. Add enough distilled water to make about 250 mls. of solution. Add 25 mls. of 1: 10 HCl solution. Dilute to the mark (500 mls.). The 5 mls. of the Benedict-Hitchcock standard solution contain 1 mg. of uric acid. This diluted to 500 mls. makes each 5 mls. contain 0.01 mg. of uric acid. In order to prepare the stronger standard proceed as follows: Repeat the above method, except that 10 mls. of the Benedict-Hitchcock standard solution are used in place of 5 mls. This gives for each 5 mls. of solution 0.02 mg. uric acid.

[4] In preparing this solution a trace (2 mls.) of concentrated ammonium hydroxid is added per 1000 mls. of solution.

Let the tubes remain in the cold water for three minutes. Stopper, invert, and transfer at once to their respective colorimeter cups. Calculation:

$$\frac{R_s\,(y)}{R_u\,(n)} = \frac{C_u\,(x)}{C_s\,(0.02\text{ mg.})}$$

Solving:

$$x = \frac{0.02\,y}{n}\text{ mgs. per 5 mls. of 1:10 blood.}$$

Or:

$$x = \frac{0.2\,y}{n}\text{ mgs. per 5 mls. whole blood.}$$

Or:

$$x = \frac{4\,y}{n}\text{ mgs. per 100 mls. whole blood.}$$

Method of Folin.—Principle: Uric acid is precipitated as silver urate, which is centrifuged from the rest of the urine. The urate is decomposed by NaCl and then colored. Procedure: Pipette 5 mls. of the blood filtrate obtained as described under N. P. N. (page 814) into a 15-ml. centrifuge tube. It is better to carry all such determinations involving the use of centrifuge in duplicates because it is necessary to balance the tubes in the machine. In the present case the second tube is afforded by the standard which is treated in the same manner as the unknown. Pipette into a second tube, similar to the first, 5 mls. of the standard uric acid solution.[1] To each tube add 7 mls. of silver lactate solution and mix by rotating the tube between the palms of the hands. Balance the two tubes against one another in the balance (Fig. 140) near the centrifuge and centrifuge for two minutes at high speed. Decant the supernatant fluid by pouring off all you can and then invert the tubes on a piece of filter-paper. Now add 1 ml. of the special sodium chlorid solution and stir with a slender glass rod having a ball point. Add 4 mls. of distilled water and stir again. Wash off the adhering liquid from the stirring-rod with a small stream of water from your wash-bottle, taking care to use as little water as possible and to maintain the same volume in the two tubes. Centrifuge again for one minute. Decant the supernatant liquid from each tube into another tube similar to those used in the Benedict method. Reject the precipitate. The rest of the method follows that of Benedict: To each tube add 1 ml. of uric acid reagent, 3 drops of lithium sulphate solution, and 2 mls. of distilled water. Rotate the tubes between the palms of the hands, and while the contents are still in motion add, from a burette, 2 mls. of

[1] Made as described for uric acid in the urine, pages 726 and 910.

the special sodium cyanid solution (Caution!). Let stand for about two minutes, place the tubes in boiling water for 1.5 minute, and then cool under the cold water-tap. Dilute to 25 mls. with distilled water, stopper the tubes, and mix by inverting them several times. Then compare the colors in a colorimeter. Calculation: Placing the standard at 20 mms.,

$$\frac{R_s\ (20)}{R_u\ (n)} = \frac{C_u\ (x).}{C_s\ (0.02)}.$$

Solving,

$$nx = 0.4$$

And

$$x = \frac{4}{10\ n'}, \text{ or } \frac{2}{5n} \text{ mgs. per 5 mls. of 1:10 blood.}$$

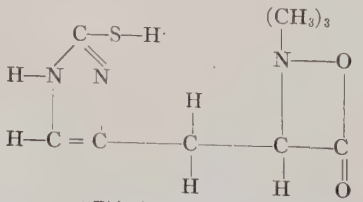
Then

$$x = \frac{80}{n} \text{ for 100 mls. of whole blood.}$$

The above method, in which substances in the urine that act upon the reagent are removed by centrifuging, has been found to obviate the cloudiness which sometimes appears when the direct method is used by some inexperienced workers. Benedict has shown[1] that human and animal blood contain a substance (or substances), 1 gram of which he has isolated, which substance is precipitated by silver lactate and reacts with the uric acid reagent. However, since the silver compound of this substance is not decomposed in the method just given, it does not vitiate the results of this method.

Method of Benedict Modified by Folin.— Principle: This method utilizes principles derived from the experiences of Benedict and of

[1] Jour. Biol. Chem., vol. 64, p. 215, 1925. Benedict discovered in 1922 that certain unknown substances were carried down with the silver urate and increased the actual value of uric acid in blood determinations. One of these substances at least has been isolated and found to be a histidin-like substance which Benedict terms thiazin, but which he finds to be identical with a previously described substance:



"Thiazin;" (Benedict) betaïn of thiohistidin. (Barger and Emins)

See Newton, E. B., Benedict, S. R., and Dakin, H. D., Science, vol. 64, page 602, 1926.

Folin, the chief procedure being the direct method of Benedict, the application of heat in order to cause a deeper color of the uric acid reagent, and the use of the blood filtrate obtained after tungstate—H_2SO_4 precipitation. On the other hand, Folin dispenses with the arsenic solution of Benedict, since heat develops an adequate amount of color. A simplified uric acid standard reagent is employed.

Reagents: See Appendix.

Procedure: If a steam bath is not immediately available, place a 500 ml. beaker, two-thirds full of tap water on an asbestos gauze over a bunsen burner and bring the water to boiling. Set up two burettes; fill one with 15 per cent. NaCN and the second with the uric acid reagent.

Into a 200 × 20 mm. pyrex test-tube[1] pipette accurately 5 mls. of the tungstic—H_2SO_4 filtrate (page 814). Add to a second, similar tube, 5 mls. of the standard uric acid solution (5 mls = 0.02 mg. uric acid). Now to each tube add 2 mls. of the cyanid solution from the burette and then 2 mls. of distilled water. Mix. To each tube add 1 ml. of the uric acid *reagent*. Mix, let stand 2 minutes, then place the tubes in the water-bath for exactly 2 minutes longer. Now cool the tubes under the cold water tap and immediately add sufficient water from your water-bottle to bring the volumes to the 25 ml. mark. Stopper, invert and compare in a colorimeter. Calculation: Set the standard at 20.0 mms. Then:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} = \frac{\text{Concentration of unknown}}{\text{Concentration of standard}}$$

$$\frac{R_s = 20}{R_u = u} = \frac{C_u = x}{C_s = 0.02}$$

$$= 80/u \text{ mgs. per 100 mls. blood.}$$

CREATININ IN THE BLOOD

Clinically, the data given by the Folin method for creatinin in the blood[2] has been found valuable in prognosis and treatment. Benedict and Behre,[3] however, believe that "no results so far available offer definite evidence of the existence of creatinin in blood." Greenwald has isolated a red compound which is responsible for the Jaffé reaction (page 367) upon which Folin's method for creatinin depends. Substances other than creatinin may give this reaction. Benedict finds that the so-called creatinin of the blood reacts more readily with picric acid than true creatinin does. He has found, also, that

[1] The standard blood tubes graduated at 25 mls. are preferable.

[2] Ibid., vol. 17, p. 475, 1914.

[3] Behre, J. A., and Benedict, S. R., Jour. Biol. Chem., vol. 52, p. 11, 1922.

amounts of pure creatinin comparable to those supposed to occur in the blood when heated with alkali no longer give the Jaffé reaction, but that blood filtrates treated in a similar manner show little change from filtrates not so treated. Again, kaolin[1] abstracts pure creatinin from blood filtrates up to amounts of 3.5 mgs. per 100 mls. of filtrate, but the "creatinin" of blood is not affected by kaolin. Lastly, although the evidence is based upon only a single experiment, Benedict found that there is no indication that "creatinin" of nephritic blood is true creatinin; the blood of a dog which had had its ureters ligated (imitating prostatic obstruction) showed no true creatinin as judged by direct zinc chlorid precipitation, although 5 mls. of the filtrate showed 9 mgs. of "creatinin" by the usual blood creatinin method.

Regarding the creatinin supposed to occur in blood, the following data have been collected:

Normal amount[1] ..... 1 to 2 mgs. per cent. whole blood

[1] In the laboratories of Jefferson Hospital the normal creatinin content of blood is taken as 1.5 mg. per 100 mls. of whole blood, based upon data accumulated by different workers over several years. The cases were checked against their histories.

Increase after feeding creatinin by mouth:[2]

Time.	Creatinin by mouth.	Blood creatinin mgs., per cent.
7/ 2/1920	None	1.04
7/10 "	1 g. single dose	2.27
7/13 "	3 gs. single dose	2.50
7/13 "	3 gs. two doses	2.12
7/17 "	5 gs. single dose	4.75
7/21 "	10 gs. single dose	7.90
7/21 "	10 gs. two doses	6.15
7/22 "	10 gs. three doses	2.14[1]

Increased in cardiorenal conditions ..... 3 to 4 mgs.
Increased in apparently normal conditions ..... Up to 3.5 mgs.
Increased in long-standing diabetes mellitus ..... 3 to 4 mgs.
Increased sometimes in intoxications with fever ..... 3.5 mgs. and lower
Increased in interstitial nephritis in amounts corresponding to the severity of the disease ..... Up to 30 mgs.
Increased in intestinal obstruction ..... Up to 10 mgs.

[1] This series of figures shows that there is probably some interdependence of true creatinin upon the so-called "creatinin of the blood."

Myers believes that a creatinin content greater than 3.5 mgs. per 100 mls. of whole blood indicates great severity and bad prognosis for the course of the disease.

[1] A highly purified clay. Compare the method of Greenwald, I., and McGuire, G., Jour. Biol. Chem., vol. 34, p. 103, 1918.

[2] After Berglund, his Table III.

The distribution of creatinin between plasma and corpuscle is shown in the following data after Berglund:

	Plasma creatinin.	Corpuscle creatinin.
Where the whole blood creatinin is 1.16 mg. per cent.....	1.17	1.17

Attention is called to the "staircase" (page 824). It will be seen that the passage of creatinin through the kidney is affected later than that of other constituents; in other words, creatinin is more easily cared for by a defective kidney than uric acid and urea.

Quantitative Method for the Determination of Creatinin in Blood.[1]—Principle: The creatinin or chromogen of the blood giving the Jaffé reaction is converted into a picrate by treating the blood with concentrated solutions of picric acid in alkaline solution The color developed is compared with that produced by treating a known quantity of pure creatinin or the zinc chlorid in the same manner.

Procedure: Pipette 25 mls. of a saturated solution[2] of pure picric acid into a 100-ml. Erlenmeyer flask and add 5 mls. of 10 per cent. NaOH solution. Agitate the contents of the flask and let stand for several minutes before using. In the meantime pipette 10 mls. of the tungstic filtrate obtained as described under N. P. N. into a 50-ml. heavy glass shaking bottle. To a second and similar bottle add 5 mls. of the standard creatinin solution (page 737), dilute with 15 mls. of distilled water. Add to the unknown (first bottle) 5 mls. of the alkaline picrate prepared above and to the standard 10 mls. Stopper the bottles and place them on the platforms of the shaking machine;[3] screw down the clamp and shake at moderate speed for two minutes. Leave longer at reduced speed if possible; however, the maximum time should not be greater than ten minutes. Make the comparison in a colorimeter.

[1] The method given here is primarily that of Folin modified according to recommendations of Benedict, Greenwald, Myers, as well as by Folin and collaborators.

[2] The solution must be thoroughly saturated at the temperature at which it is to be used. This is best done by warming a solution of picric acid that has stood over a residue of the crystals for a week or more and has been shaken at intervals. While warm, shake the bottle vigorously, let any undissolved crystals settle, and decant into a beaker a small amount of supernatant liquid which is to be used in the determination. Then cool this solution to room temperature under the cold water-tap immediately before use. A still better plan is to maintain in a shaking machine a supersaturated solution of picric acid at all times and to draw from it at the time that a determination is to be made. The method for purifying picric acid for these determinations is given in the Appendix, taken from the Jour. Biol. Chem., vol. 54, p. 239, 1922. Folin's method is given in the same journal, vol. 28, p. 349, 1917.

[3] The directions are for the International Equipment Co. centrifuge with head shaker. Any shaking device may be used.

Calculation:

$$\frac{R_s\ (20)}{R_u\ (n)} = \frac{C_u\ (x)}{C_s\ (0.015\ mg.)}.$$

Then

$$x = \frac{3}{10\ n} \text{ for 10 mls. of 1:10 solution.}$$

$$x = \frac{30}{n} \text{ per 100 mls. whole blood.}$$

CREATIN

Creatin is a normal constituent of blood, average normal 6.0 mgs. per 100 mls. of whole blood. Marked variations characteristic of disease have not been observed.[1] It is measured by first determining the preformed creatinin according to the method just discussed and then converting the creatin to creatinin by the same method that was used for creatin in the urine. The method is given in Folin's Manual, page 247, edition of 1922.

THE AMINO-ACIDS IN THE BLOOD

The amino-nitrogen of blood is the largest portion of the non-protid nitrogen with the exception of the urea nitrogen. Following a meal the amino-acid nitrogen rises from an average normal of 5.7 to 7.8 mgs. per 100 mls. of whole blood to much higher figures (over 12 mgs.), depending upon the amount of protid ingested. Amino-nitrogen is then largely exogenous in character, and for this reason is not significant clinically. There is a relatively large destruction of amino-acids in the liver and other tissues, both (1) of those derived from the food and entering the liver by way of the portal vein; and (2) of those which have been "pushed out" of the tissues by the amino-acids which escape destruction in the liver. Whether there is a need for amino-acids in the tissues or not, these acids are destroyed in the liver to a certain extent. Even after a time of fasting, when the concentration of amino-acids in the tissues is low, if a protid diet is resumed the liver allows only a relatively small amount of the amino-acids to pass through unchanged; these are, of course, greedily absorbed by the tissues. There is no storage of amino-acids in the tissues; after they have remained in a tissue for a period which varies with the kind of tissue and the amino-acid concentration, they pass again into the blood-stream and circulate throughout the body. If they reach the liver they are destroyed. We do not know whether they pass to other organs and find lodgment or not. It is known,

[1] Looney (page 732) finds that creatin varies with muscle tone.

however, that one tissue may be called upon to provide amino-acids for another tissue.

Pathologically, amino-nitrogen rarely rises in the blood. "The deaminization process appears to be such a fundamental process that one cannot expect to find many pathological conditions in which the amino-nitrogen of the blood filtrates will vary very much from the normal."[1] Even during severe nephritis when the N. P. N. of the blood rises to over 300 mgs. per 100 mls. of blood, there is no characteristic increase in amino-nitrogen. However, in extensive destruction of liver tissue, as in acute yellow atrophy, amino-nitrogen rises to about 30 mgs., according to the severity of the disease and the individual factors concerned. Two factors conspire to cause this increase: (1) Great destruction of tissue and (2) loss of power of deaminization.

Methods for Determining Amino-nitrogen in Blood.—Two methods are available: (1) The gasometric method of Van Slyke (page 294) and (2) the colorimetric procedure of Folin. The results seem to be practically identical.

Gasometric Method of Van Slyke.—The general principles of this method and its application to certain fluids have already been given on page 291. In the case of blood it is necessary to obtain a protid-free filtrate. This is done by the following method, although the tungstate filtrate described for N. P. N. on page 814 may be used.

Precipitation of the protid for the Van Slyke procedure: (*a*) For whole blood: Pipette 10 mls. of oxalated blood into a 250-ml. volumetric flask. Add about 100 mls. of distilled water. Add 20 mls. of 10 per cent. mercuric sulphate solution, $HgSO_4$. Mix and let stand about ten minutes. Then dilute to the mark (250 mls.), stopper, invert, agitate the contents, and filter through a fluted filter (Fig. 218). The filtrate may be poured back over the residue in the funnel if there is discoloration. The filtrate is always, however, pink tinged. The substance responsible for this color does not interfere with the procedure.

(*b*) For plasma; also for serum: Pipette 8 mls. of the plasma into a 200-ml. volumetric flask and add about 50 mls. of distilled water. Add 15 mls. of the mercuric sulphate solution. Agitate the contents of the flask. After about eight minutes dilute to the mark with distilled water, mix, let stand about five minutes, and filter as in (*a*).

Procedure: From 2 to 10 mls. of the filtrate from either (*a*) or (*b*) are used in the larger form of the Van Slyke apparatus and from 0.5

[1] Folin, O., cited on page 26; his page 476.

to 2 mls. in the micro-form. The various steps are described on page 294. The calculation is the same.

The Colorimetric Method of Folin.—Principle: Comparison of the red color produced by bringing the amino-acids in contact with a quinon compound (sodium β-naphthoquinon-sulphonate), with the color developed by a known quantity of glycin; cystin, leucin, tryosin, aspartic acid, phenylalanin, or glutamic acid may be substituted. A proper degree of alkalinity must be maintained by means of sodium carbonate solution. The color is intensified by adding an acetate solution, which serves also to inhibit turbidity. Any excess quinon is destroyed by adding sodium thiosulphate solution.

Procedure:[1] Pipette 5 mls. of the tungstic filtrate obtained for N. P. N. (page 814) into a 200 × 20 mm. test-tube. Into a similar tube pipette 1 ml. of the standard amino-acid solution and add about 3 mls. of distilled water. Now add to each tube 1 drop of a 0.25 per cent. phenolphthalein solution as indicator. Add 1 ml. of 1 per cent. Na_2CO_3 solution to the standard amino-acid solution and to the unknown add the carbonate drop by drop until the colors in the two tubes match. Add another 5-ml. amount of distilled water to the standard, thus making its volume twice that of the unknown. Add 2 mls. of a freshly prepared solution of sodium-β-naphthoquinon-sulphonate. To the other tube (the unknown) add 1 ml. Roll each tube between the palms of the hands in order to mix the contents and then leave the tubes in the dark (locker) for at least twenty-four hours. To the standard add 2 mls. of the special acetate solution, and to the unknown, 1 ml. Then add to the standard 2 mls. of 4 per cent. sulphate thiosulphate and to the unknown 1 ml. From a Folin-Wu blood pipette (Fig. 251) or a burette add to the standard tube 14 mls. of distilled water and to the unknown 7 mls. The volume for the standard should now be 30 mls. and that of the unknown, 15 mls. Stopper the tubes, invert, and half fill each cup of a colorimeter. Set the reading of the standard at 20 mms. Make the comparison.

Calculation: n being the reading of the unknown, $\frac{140}{n}$ mgs. is the amino-acid nitrogen per cent. whole blood.

Undetermined Nitrogen of the Blood.—After the various fractions of non-protid nitrogen have been determined (urea, uric acid, etc.) it is found that their sum is less than the non-protid nitrogen. It is not known at present what substances are responsible for this

[1] The reagents are described in the Appendix.

deficit. In intestinal obstruction (page 813), a marked increase of non-protid nitrogen occurs. The general appearancs resembles albumose intoxication and doubtless the polypeptids are responsible for some of the undetermined nitrogen.[1]

It is possible that this fraction also contains materials of a toxic nature, for example, amins, as Looney has pointed out[2] a correlation between the toxicity of blood to the growth of seedlings of *Lupinus albus* and the amount of undetermined nitrogen. There is an increase in this fraction in the case of patients suffering from marked depressions characterized by apathy, listlessness and lassitude.[3]

BLOOD SUGAR

Little is known of blood sugar. Determinations are made and the results are interpreted as glucose. That some of the reducing substance of the blood consists of another form of glucid is evident from the occurrence of lactose in the urine of lactation; of pentoses, polyglucids and glucidtemns, the "normal" sugar of urine (page 768). Diabetics in which insulin does not lower blood "sugar" also illustrate this point. On the other hand, qualitative tests, such as fermentation, the osazone, etc., demonstrate not only that glucose is present, but that it is the important glucid. Van Slyke[4] reviewed attempts made to identify glucose. As to yeast fermentation, Wolf[5] warns that similar conclusions have been drawn for urine "glucose," but no carbon dioxid is demonstrable in such "fermentations." Van Slyke also permitted blood to glucolyze at normal temperature. The results were uniform by the two methods. *Glucose is* 0.07 *to* 0.09 *g. of the total reducing substances of the blood* (0.10 *g. per* 100 *mls. blood*), *leaving* 0.01 to 0.03 *g. as non-fermentable residual substance.* Insulin causes the disappearance of glucose, leaving unaffected the residual substance. In glomerular nephritis the increase in blood sugar is due to glucose.

[1] For a review see Myers, V. C. Chemical Changes in the Blood and Their Cinical Significance, Physiol. Revs., vol. 4, page 274, 1924.

[2] Looney, J. M. and Macht, D. I. (page 492). The Relation between the Undetermined Nitrogen of the Blood and Its Toxicity to Lupinus Albus Seedlings, Jour. Biol. Chem., vol. 63, page lx (Proc.), 1925.

[3] See Looney, J. M. A Biochemical Study of the Blood in mental Disorders, Amer. Jour. Psychiatry, vol. 4, page 29, 1924.

[4] Hiller, A., Linder, G. C., and Van Slyke, D. D., Jour. Biol. Chem., vol. 64, p. 625, 1925.

[5] Lund, G. S., and Wolf, C. G. L., (Addenbrooke's Hospital, Cambridge, England), Biochem. Jour., vol. 19, p. 538, 1925.

The distribution of glucose between the corpuscles and the plasma has been investigated by Dowds,[1] who finds that in venous blood, more sugar occurs in the corpuscles, but in capillary blood, more is found in the plasma.

Method for Quantitative Determination of Blood Sugar.—Two chief methods exist. The normal figures in use are those determined by Strouse,[2] who adapted the original Bertrand technique,[3] modified by Kovarski.[4] The method of Bang,[5] used in Europe, gives reliable results. The principle of Bang's method—iodometrical determination of reduced cuprous salts—was applied by Shaffer[6] and later by Tervaert[7] to blood sugar studies. These methods are copper reduction methods.

The two methods described in the following pages are colorimetric methods suited to rapid clinical work. The method of Benedict seems to give higher readings than that of Folin. However, Benedict's method is widely used, especially in laboratories which supply vacuum tubes (Figs. 237 and 238) to their clients; these tubes contain picric acid solution which causes the precipitation of the protids of the blood and at the same time supplies the necessary substance for making the colorimetric determination. An anticoagulant is unnecessary when these tubes are used.

Method of Benedict.[8]—Principle: The principle is given under the subject Chemistry of the Glucids (page 163). Picric acid is converted into picramic acid in a process of reduction, the sugar becoming oxidized by oxygen from the nitro-groups of the tri-nitro-phenol (picric acid). A modification of the technique given by Benedict

[1] Dowds, J. H. (Johannesburg, South Africa) Biochem. Jour., vol. 20, page 1173, 1926.

[2] Strouse, S. (Professor of Medicine, Northwestern University Medical School, Chicago, Illinois). See Johns Hopkins Hospital Bull., vol. 26, p. 211, 1915.

[3] Bertrand, G. (Professor in the University of Paris, France), Bull. de la Société Chimique de Paris, vol. 35, p. 1285, 1906.

[4] Kovarski, A. (German clinician), Deutsche, Med. Wochenschr., vol. 39, p. 1635, 1913.

[5] Bang, I. (German biochemist), Die Blutzucker, Wiesbaden, 1913.

[6] Shaffer, P. A., (p. 522), and Hartmann, A. F. Jour. Biol. Chem., vol. 45, p. 349, 1921.

[7] Tervaert, D. G. C. (Utrecht, The Netherlands), Biochem. Jour., vol. 19, p. 541, 1925.

[8] Lewis, R. C. (Professor of Biochemistry, University of Colorado, Boulder), and Benedict, S. R., Jour. Biol. Chem., vol. 20, p. 61, 1915; vol. 34, p. 203, 1918; vol. 37, p. 503, 1919. For a method adapting this procedure to small amounts of blood see Smith, M. (Joslin's Clinic, Deaconess Hospital, Boston), Jour. Lab. and Clin. Med., vol. 7, p. 364, 1922.

Fig. 244.—The Lewis-Benedict blood sugar tube.

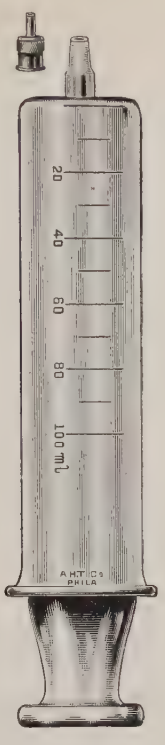


Fig. 245.—The Luer type syringe. For needles used with this syringe see Fig. 240.

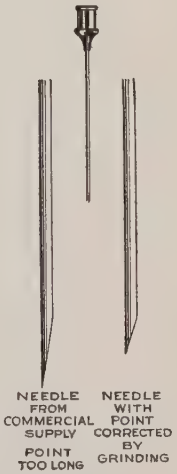


Fig. 246.

Fig. 247.—The combination tube proposed by Nash, suitable for the Lewis-Benedict, Myers-Bailey, and other blood sugar and nitrogen determinations.

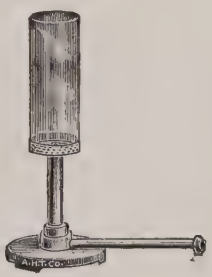


Fig. 248.—"Micro"-burner used in many biochemical procedures. A mica chimney may be used, as shown, to avoid draughts.

has been made in the Mt. Sinai Hospital Laboratories, New York, N. Y., by A. A. Epstein (Jour. Amer. Med. Assoc., vol. 63, p. 1667, 1914). Procedure: For whole blood: Blood is withdrawn from a vein[1] by means of a hypodermic needle (20 gauge, 1.25 inch Luer type, see Fig. 240). The needle is then thrust through a rubber tube attached to an Ostwald-Folin pipette (2 mls.) which contains in its tip some powdered, crystalline potassium oxalate. Enough blood is injected in this manner into the pipette to make more than 2 mls. with the oxalate. Adjust the meniscus of the blood column

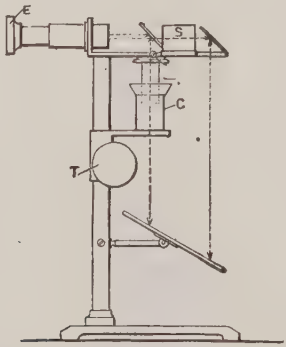


Fig. 249.—Bock-Benedict type of colorimeter. The eye, applied at *E*, sees simultaneously an image due to light from (1) the standard in the small rectangular cell *S* and (2) the unknown in the cup *C*. By moving the unknown up and down by means of the milled head, *T*, the field is finally made uniform.[2]

in the pipette to the mark; the pipette delivers exactly 2.0 mls. of blood. Now transfer the 2 mls. of blood to a 25-ml. volumetric flask containing 5 mls. of distilled water. Mix. Add 15 mls. of the special alkaline solution of picric acid[3] and 1 drop of caprylic alcohol in order to prevent foaming. Add distilled water to the mark, stopper, invert the flask, and agitate the contents. Filter through a dry filter into a small beaker. Pipette 8 mls. of the filtrate into a 200 × 20 mm. Pyrex test-tube; it is convenient to use tubes graduated at 12.5 and 25 mls. (Figs. 244 and 247). Add 2 mls. of the

[1] Superficial blood-vessels give blood that is more nearly arterial in character than purely venous blood, and for some purposes it is desirable to withdraw blood from the finger-tip, ear-tab, vein from the back of the hand, etc. See Foster, G. L., Jour. Biol. Chem., vol. 55, p. 291, 1923.

[2] In calculating determinations made by the Bock-Benedict colorimeter, it is necessary to know the depth through which the standard solution, in the cup *S*, is viewed; in order to accomplish this determination, fill both cups with the standard solution and match the colors; the reading of the standard and of the "unknown" are, of course, the same and the figure obtained must be used in Beer's formula for the reading of the standard.

[3] Appendix.

special picric acid solution and 1 ml. of sodium carbonate solution.[1] Stopper the tube with non-absorbent cotton and leave it in a boiling water-bath for ten minutes or longer. Cool the tube under the cold water-tap. Dilute to the mark with distilled water. The dilution is made according to the probable amount of sugar in the specimen. If it is large, dilution is made to 25 mls., judging from the depth of color, otherwise to 12.5 mls. Compare in a colorimeter with a standard picramic solution or, better, with a solution of glucose (0.64 mg. glucose; 0.00064 g.): Place 4 mls. of distilled water in a 200 × 20 mm. test-tube and add 0.64 mg. of glucose.[2] Add 4 mls. of the special picrate solution and 1 ml. of the sodium carbonate solution. Leave in the boiling water-bath for ten minutes along with the unknown.

Calculation:

$$\frac{R_s\ (20)}{R_u\ (n)} = \frac{C_u\ (x)}{C_s\ (0.64\ mg.)}$$

Solving:

$$x = \frac{12.8}{n} \text{ mgs. for 8 mls. of filtrate,}$$

Or:

$$x = \frac{40}{n} \text{ mg. for the 2 mls. whole blood, or } \frac{2}{n} \text{ g. per cent.}$$

If the standard is placed at any figure other than 20 mms., then:

$\frac{R_s}{R_u}$ divided by 10 gives the per cent. of blood sugar in whole blood.

If the blood has been collected in a vacuum tube, the tip of the tube is broken; otherwise the procedure is like that given above.

Method of Folin.[3] —Principle: The sugar of the blood is boiled with a copper solution; the copper is reduced as in Fehling's and other methods. A phosphomolybdic acid solution is then added; this molybdenum compound reacts with the reduced copper (cuprous oxid or hydroxid), producing an intense blue like that noted in the determination of uric acid.

Procedure:[4] Pipette 2 mls. of the blood filtrate used in the previous determinations (page 814) into a Folin special blood-tube (Fig. 250[5]). Prepare the standards: Pipette into a second tube,

[1] Twenty gs. of Na_2CO_3 made up to 100 mls. with distilled water.

[2] The glucose may be kept in a solution in water, protected from bacterial action and of such strength that an aliquot part, say 1 ml., contains 64 mgs. glucose.

[3] Folin, O., and Wu, H. (page 814), Jour. Biol. Chem., vol. 41, p. 367, 1920.

[4] For reagents see the Appendix.

[5] This form of tube has been used for many years in quantitative analysis to suppress reoxidation by the oxygen of the air. Reduction takes place in the bulb of the tube and the constricted portion does not permit air to enter the bulb, except an extremely small amount during ebullition.

similar to the one just mentioned, 2 mls. of the standard glucose solution containing 0.2 mg. (weaker) and into a third similar tube 2 mls. of the standard glucose solution containing 0.4 mg. (stronger standard). These standards give a range of blood sugar from 70 to 400 mgs. per 100 mls. of whole blood. Now to each of the three tubes add 2 mls. of the alkaline copper solution.[1] Leave the three tubes in a water-bath at 100° C. for six minutes. Then transfer them to a beaker of cold water and leave for three minutes. Add to each tube 2 mls. of the phosphomolybdate solution. When the cuprous oxid has dissolved add water to make the total volume 25 mls.; it is convenient to have these tubes graduated at this point (Fig. 250). Stopper, invert, mix, taking care that the contents of the bulb are thoroughly mixed with those of the main portion of the tube.

Fig. 250.—Folin blood-sugar tube. The reducing reaction proceeds in the small bulb and oxygen is prevented from influencing the procedure on account of the constriction.

Fig. 251.—The Folin-Wu blood pipette, capacity 15 mls.

Calculation: If the standard is placed at 20 mms., then:

$$\frac{R_s\ (20)}{R_u\ (n)} = \frac{C_u\ (x)}{C_s\ (0.2\ mg.)}$$

Solving:

$$x = \frac{4}{n} \text{ per 2 mls. of 1:10 blood,}$$

[1] In the making of these tubes it is impossible to maintain uniformity in size of the bulb. The solution must fill the bulb, and it must intrude upon the constricted portion for half its distance. If, after adding the various solutions described above, the bulb is not full, or is too full, it is best to reject the tube and use another.

Or:

$$x = \frac{2000}{n} \text{ mgs. per 100 mls. whole blood.}$$

For the stronger standard the result is obtained by the equation $X = \frac{4000}{n}$ mgs.

Method for Finger-tip Blood.[1]—Principle: Application of the Folin-Wu procedure, just described, to minute amounts of blood, involving about 100 mgs. of blood-sugar in 0.05 to 0.1 ml. whole blood. Procedure: Dip a pledget of cotton in 70 per cent. ethanol and bathe the tip of the finger thoroughly, followed by ether to remove the fat. Stab the tip with a sterile, new steel pen-point or blood-lance. Using a special pipette, provided with mouthpiece and rubber suction tube, similar to an ordinary hemacytometer pipette, but graduated at 0.05 and 0.1 ml., draw up blood past the mark selected and, by means of filter-paper, withdraw blood until the meniscus is opposite the proper mark. If the blood is supposed to be normal, use the 0.10 quantity; if hyperglycemia, the 0.05 ml. mark. Transfer the blood to a small tube graduated at 2 mls. and containing 1.5 ml. distilled water, in order to cause hemolysis as in the Folin method. Draw the water into the pipette and expel it several times, to insure thorough mixing and quantitative results. Precipitate the protids by adding to the tube graduated at 2 mls. 0.1 ml. of 10 per cent. sodium tungstate solution and 0.1 ml. of two-thirds normal sulphuric acid.[2] Roll the tube between the palms of the hands for mixing and continue until the proper brown color described in the Folin procedure (page 814) is obtained. Make up the volume to the mark (2 mls.) with distilled water and mix again. Let stand for five minutes and then centrifuge for two minutes. Pipette the supernatant fluid from the residue into a 10-ml. volumetric cylinder. If the amount of blood taken for analysis was 0.10 ml., the supernatant fluid should be 1.4 ml., other-

Fig. 252.—Kramer-Gittleman modification of the Folin-Wu blood-sugar determination for small amounts of blood.

[1] Kramer, B., and Gittleman, I. F. (Johns Hopkins University, Baltimore, Md.), Jour. Amer. Med. Assoc., vol. 81, p. 1171, 1923.

[2] Appendix. Also page 910.

wise 1.5 ml. is obtained. Transfer the fluid to a Folin-Wu sugar tube (Fig. 252) graduated at 6 mls. as well as 12.5 and 25 mls. To two similar tubes add 1 ml. of the standard glucose solution containing 0.1 mg. per ml.[1] Add to each tube 2 mls. of the alkaline copper solution of the Folin method and leave the three tubes in a boiling water-bath for six minutes. Transfer to a cold water-bath for three minutes. To each tube add 2 mls. of the molybdate solution. Insert a rubber stopper into each tube, invert, and transfer to the cups of the colorimeter. Set the standard at 15 mms. Calculation:

$$\frac{R_s\ (15)}{R_u\ (n)} = \frac{C_u\ (x)}{C_s\ (0.1)\ \text{mg.}}$$

Solving:

$$x = \frac{1.5}{n}\ \text{mgs. per 0.07 ml. blood, or}$$

$$x = \frac{15}{n} \times \frac{1}{10} \times \frac{1000}{0.07}\ \text{mgs. sugar in 100 mls. blood.}^{2}$$

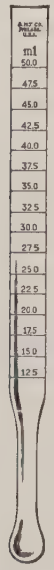


Fig. 253.—The Rothberg-Evans modification of the Folin sugar tube for blood sugar.

Fig. 254.—The Myers-Bailey tube for blood sugar.

Critique on the Folin Procedure.—Various criticisms have been urged against the methods outlined above,[3] but in the author's experience strict adherence to the specifications laid down by Folin and collaborators in their various papers will obviate the alleged

[1] If the blood is considerably hypoglycemic, make a standard containing 0.5 ml. of the standard solution.

[2] Two new methods for blood sugar have been described:

Folin, O. The determination of sugar in blood and in normal urine. Jour. Biol. Chem., vol. 67, page 357, 1926.

Benedict, S. R. The estimation of sugar in blood and normal urine. Jour. Biol. Chem., vol. 68, page 759, 1926.

[3] Rothberg, V. E., and Evans, F. A. (Singer Research Laboratory, Pittsburgh, Penna.), Jour. Biol. Chem , vol. 58, p. 435, 1923, is one such.

errors. If, however, it is preferred to modify the technique, that change advocated by Rothberg and Evans suggested in the article cited should be given first attention. The special tube (Fig. 253) advocated is designed to permit dilution of the unknown solution, after the addition of the molybdate solution, until the color of the unknown is similar to that of the standard. The higher the concentration of glucose, the farther the observed readings on the colorimeter depart from the theoretical readings. The calculation for the use of the Rothberg-Evans modification differs from that of the Folin-Wu method only in that the dilution factor must be included. If the standard be placed at 20 mms., the standard being diluted to 25 mls., then:

$$\frac{20}{n} \times 100 \times \frac{\text{dilution of unknown}}{25} \text{ gives the mgs. per cent.}$$

Or, simplified:

$$\frac{80 \times \text{dilution of unknown}}{\text{Reading of unknown}}.$$

Myers'[1] *Method.*—Principle: Modification of the Benedict method (page 000), the principal variations being of the nature of that just mentioned as a modification of Folin's method, namely, dilution of the unknown to approximate the color of the standard; the special tube (Fig. 254) is used as a convenient method of diluting to a known degree.

Procedure: Into a 20-ml. centrifuge tube resembling an ordinary test-tube add 8 mls. of distilled water and 2 mls. of well-stirred oxalated blood or plasma. Mix by means of a delicate stirring-rod and add about 0.5 g. of dry picric acid in order to cause precipitation of the blood protids, as well as to saturate the solution with the acid. When the solution becomes homogeneous in consistency and color, throughout, centrifuge for three minutes. Place a small funnel provided with folded paper in a dry test-tube in your rack and filter the supernatant liquid in the centrifuge tube. Pipette 3 mls. of the clear filtrate into a test-tube graduated as shown in Fig. 254. Add 1 ml. of saturated (22 per cent.) sodium carbonate solution and leave the tube in a boiling water-bath for about half an hour. Making the standard: Into a second tube similar to the one just described pipette 3 mls. of the standard glucose solution (0.02 g. per cent. picric acid solution); add 1 ml. of the saturated Na_2CO_3 solution. Leave in the bath together with the tube containing the unknown solution. After

[1] Myers, V. C., and Bailey, C. V. (Fig. 236 and New York Post-Graduate Medical School, New York, N. Y., respectively), Jour. Biol. Chem., vol. 24, p. 147, 1916.

there has been a maximum development of the brownish colored picramic acid,[1] which is obtained certainly within the thirty-minute period and frequently within fifteen to twenty minutes, transfer the tubes to a beaker containing cold water. When the temperature of the contents of the tubes reaches room temperature, dilute the standard to exactly 10 mls. and then dilute, carefully, the unknown to the nearest calibration (10, 15, 20, etc.) that is possible in order to have

Fig. 255.—Method of securing blood from heart of a guinea-pig. Blood for class use may be obtained in this manner. (From Kolmer, Infection, Immunity, and Biologic Therapy.)

the colors of the unknown and standard nearly match. Set the standard at 15 mms. Calculation: The calculation is like that of the Rothberg-Evans' method (page 847). Myers and Bailey give the following scheme for calculation:

$$\text{Blood sugar in gs. per cent.} = \frac{R_s \times \text{dilution of unknown} \times 0.1^2}{R_u \times 10}.$$

Gasometric Method for the Determination of Glucose.[3]—Method of Van Slyke and Hawkins.—Principle: Using the manometer (Fig. 390),

[1] Page 163.

[2] Baumann, E. J. (Montefiore Hospital, New York, N. Y.), Jour. Lab. and Clin. Med., vol. 7, p. 357, 1922, has modified the Folin-Wu procedure for small amounts of blood.

[3] Method of Van Slyke and Hawkins. The author is under obligation to Dr. Van Slyke for the procedure given above.

the nitrogen gas derived from excess potassium ferricyanid over that reduced by the unknown sugar is determined and the sugar is calculated by difference. The excess ferricyanid is treated with hydrazin, $H_2N\text{-}NH_2$ which reacts as follows:

$$4K_3FeCy_6 + H_2N\text{-}NH_2 + 4NaOH \text{—} 4K_3NaFeCy_6 + 4H_2O + N_2.$$

Reagents:

Potassium ferricyanid solution:

I.	Potassium ferricyanid	8 gs.
	Sodium carbonate, dry	150 "
	Distilled water to make	1000 mls.

Hydrazin solution:

II.	Sodium hydroxid, 40 per cent. solution	50 mls.
	Hydrazin solution, saturated solution of hydrazin sulphate, 2 gs. to 50 mls. H_2O at 20° C	50 "

Procedure: Pipette 2 mls. of the Folin-Wu blood filtrate (page 814) into a 150 × 10 mm. test-tube and add 1 ml. of the ferricyanid solution (I). Leave the tube in a boiling water-bath for 4 minutes. Cool under the tap to room temperature and then saturate the contents with air by stoppering the tube and shaking it violently for at least one minute. Now place 2 mls. of the hydrazin solution (II) in the chamber of the apparatus[1] via the small burette above cock b. By means of the special rubber-tipped pipette (Fig. 129, p. 390) pipette 3 mls. of the contents of the test-tube into the burette and thence into the mixing chamber. The reduction of the ferricyanid by the hydrazin is nearly instantaneous. Evacuate the chamber, shake the apparatus one minute and then reduce the volume of gas to 0.5 ml. Read the pressure on the manometer; this is p_1. Conduct a control without glucose, after discarding the solution. Read the pressure as p_0;

[1] The apparatus should stand unused full of cleaning solution (page 902). The procedure is: Close cocks e and b. Fill the graduated burette above cock b with the cleaning solution. Then open cock e to permit egress of any gas. When ready to use, pipette the cleaning solution from the burette, or drain into the mixing chamber below cock b by opening this cock and forcing the solution out by way of the side-tube, which is done by elevating the mercury reservoir and turning cock e appropriately. Add water to the burette and wash by proper manipulation of cocks b and e. After each determination, the cleaning may be done as follows: Eject the remaining solution through the side tube. Drop the level of the mercury to level "A cc." Add about 1 ml. of normal solution of lactic acid and 10–15 mls. of distilled water to the burette and thence to the mixing chamber. Leaving cock b open, shake the apparatus for about 20 secs. Eject the gases. The apparatus is now ready for use, as the small amount of liquid adhering is gas-free and will not affect the determination.

this control has to be done but once for the set of determinations. The *calculation* is as follows: Pressure drop caused by 1 mg. glucose is:

256 mm. mercury at 15° C.
261 " " " 20
265 " " " 25
269 " " " 30

Correction for temperature: If, in the interval between the determination and the control, the temperature changes, add to or subtract from p_0 the following amounts for each degree Centigrade rise or fall:

If the change occurs between 15°–20°, 1.5 mm.
" " " " " 20 –25 , 2.0
" " " " " 25 –30 , 2.4

Glucolysis, or the Destruction of Glucose in the Blood.—When blood is shed, there is a rapid destruction of glucose in the specimen. Earlier workers, like Claude Bernard (page 376), Lèpine, and others attempted to learn the nature of the action and the latter postulated a special enzyme. We now know that whatever the agent is, it resides in the corpuscle. Irving[1] has analyzed the factors and finds that glucolysis occurs on the surface of the erythrocyte, that the optimum reaction is *p*H 8.5, that not glucose alone, but also fructose and mannose are degraded, while galactose in the adult rabbit is very slowly affected but is to a greater extent in the young; moreover, rhamnose is unchanged; finally, that it does not seem necessary to have an organic phosphate present for the action to occur, which is corroborative of Morgulis and Barcus' opinion.

BLOOD CHOLESTEROL

Fourteen hundredths g. per 100 mls. of whole blood is the normal average concentration of cholesterol in human blood. Gardner[2] finds an average somewhat higher (0.172 g. per cent.). It plays an important rôle in the blood. It prevents profound changes in osmotic relations in the plasma. It is a carrier of fatty acids. Bloor[3] finds that the unsaturated fatty acids of the plasma of blood are largely held as cholesterol esters.[4] These substances seem to be

[1] Irving, J. T. (Cambridge, England), Biochem. Jour., vol. 20, pages 613 and 1320, 1926.

[2] Gardner, J. A. and Gainsborough, H. Biochem. Jour., vol. 21, page 130, 1927.

[3] Bloor, W. R. (Fig. 94), Jour. Biol. Chem., vol. 63, p. xlv (Proceedings), 1925.

[4] The esterification of cholesterol with a fatty acid is by way of the secondary alcohol attached to one of the aromatic rings (see page 217).

absent in the fetal blood. Since it is the unsaturated fatty acids that are concerned with the metabolism of fat, the importance of cholesterol is evident. Cholesterol is free in the corpuscle (erythrocyte). The following table[1] gives characteristic variations of blood cholesterol:

Factors lowering blood cholesterol:

Characteristic of no disease (Denis).

Elevated body temperatures and perhaps external temperatures, since tropical natives have low cholesterol.

Cancer, in late stages only (Denis).

Anemia and cachexia,[2] as in beriberi and muscle dystrophies.

Factors increasing blood cholesterol:

High lipid diet.

Diabetes mellitus (see below).

Nephrosis, but none in nephritis (Bloor).

Pregnancy (see below).

Hepatic disorders, especially obstruction of the biliary apparatus.

Hemiplegia.

In diabetes mellitus "cholesterol is distinctly related to the prognosis."[3] In earlier and less involved cases it occurs proportionately to blood sugar:[4]

	Grams cholesterol.	Grams blood-sugar.
Normal average of 23 bloods	0.22	0.10
Mild diabetes, average of 32 bloods	0.24	0.17
Moderate diabetes, average of 37 bloods	0.26	0.26
Severe diabetes, average of 55 bloods	0.41	0.23

The following table gives the value of cholesterol in expectancy of life in diabetes mellitus in which neither diet nor insulin are of avail:[1]

Duration of life observed in patients.	Number of cases.	Cholesterol per cent. whole blood.
0.8 year	4	0.8 to 1.5 gm.
1.9 years	27	0.43 to 0.79 gm.
2.4 years	19	0.32 to 0.42 gm.
4.0 years and over	73	0.31 and less

[1] From Myers, after Rothchild and Wilensky (Mt. Sinai Hospital, New York, N. Y.), Amer. Jour. Med. Sci., vol. 156, p. 239, 1918. The reference is to page 411.

[2] McCrudden, F. (Robert B. Brigham Hospital, Boston, Mass.), Jour. Amer. Med. Assoc., vol. 70, p. 1216, 1918. (Footnote 3.)

[3] Joslin, E. P., The Treatment of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1923.

[4] Table from Joslin just quoted, Table 83, page 192. The figures are for whole blood in grams per 100 mls. blood.

[5] Joslin, Table 87, page 199.

During pregnancy cholesterol biliary calculi are frequently found in women in the Occident,[1] and this condition is accompanied by hypercholesterolemia.[2]

In *eclampsia* high cholesterol seems to be correlated with accompanying nephritis. The figures for cholesterol may reach 278 in eclampsia or 334 in renal disease.

Method for the Determination of Cholesterol.[3]—*Method of Bloor.* Principle: Cholesterol esters are hydrolyzed and the free cholesterol derived from the esters, together with that which was free in the blood (especially in the corpuscles), is determined by separating it from other portions of the blood through absorption by solvents, and treating it with acetic anhydrid, as in the Liebermann-Burchard test for cholesterol (page 216). Procedure: Pipette 5 mls. of whole blood obtained as described on page 67 into a 100-ml. volumetric flask that has previously been provided with 75 mls. of a mixture of "absolute" ethanol 3 parts and anhydrous ether 1 part. The ether must be redistilled if the commercial product is used. Rotate the contents of the flask so that the blood will not clump together. Now immerse the flask in the boiling water-bath while maintaining the rotary motion until the contents begin to boil. Cool, make up to the mark with the alcohol-ether mixture, stopper loosely, mix, and filter through dry filter-paper into a small Erlenmeyer flask. When about 25 mls. have passed through the paper pipette 20 mls. of the filtrate into a Whitall Tatum "Non-Sol"[4] Erlenmeyer flask. Add 0.1 ml. (2 drops) of 50 per cent. NaOH solution made from metal sodium and distilled water, and leave the flask in the boiling water-bath, rotating the contents at frequent intervals and noting when the residue is concentrated to about 2 drops of liquid; the whole bottom of the flask must be kept moist with the liquid contents of the flask. At this stage there should be no odor of alcohol. Cool. Add 0.1 ml. solution of sulphuric acid (1 part H_2SO_4 and 3 of H_2O). Mix. The contents of the flask must now be alkaline; if the acid used has been strong enough to render the contents of the flask acid to litmus paper,

[1] For unknown reasons Oriental peoples do not have high cholesterol in the blood, nor is there a prevalence of gall-stones in either sex.

[2] Bunker, C. W. O., and Mundell, J. J. (Medical Corps, U. S. Navy, Washington, D. C.), Jour. Amer. Med. Assoc., vol. 83, p. 836, 1924.

[3] For another method see Leiboff, S. L. (Lebanon Hospital, New York, N. Y.), Jour. Biol. Chem., vol. 61, p. 177, 1924.

[4] This glass is used owing to its refractoriness toward highly caustic solutions, such as the ones used in this process. The best form of vessel is the wide-mouth "carbon dioxid" beaker, capable of being stoppered (Fig. 215). The glass in question is obtainable from the Whitall Tatum Company, 410 Race Street, Philadelphia, Pa.

add dilute sodium carbonate solution until they become alkaline. Masses of sodium sulphate will now appear in the flask, and these must be kept from forming aggregates by striking the flask against the palm of the hand. Return the flask to the water-bath and leave until no more moisture adheres to the walls of the vessel.

Dissolving the cholesterol: Cool the flask and add 10 mls. of chloroform. Mix and let stand for about ten minutes; if any substance adheres to the sides of the flask, rotate the contents. Fit a hardened filter-paper into a small funnel and pour the chloroform into it. Add to the residue in the flask 5 mls. of chloroform and repeat the above procedure, uniting the filtrates in which all of the cholesterol from the 20 mls. of blood filtrate is found.

The determination: Leave the flask containing the filtrate in the water-bath until the volume of the liquid in it is about 2 mls. Then transfer the contents to a small volumetric cylinder (10 mls.) that can be stoppered. Rinse the flask with a few drops of chloroform and add these rinsings to the cylinder until you have a volume of 5 mls. Add 1 ml. of acetic anhydrid, $\begin{matrix} H_3C.CO \\ \diagdown \\ O \\ \diagup \\ H_3C.CO \end{matrix}$, and 0.1 ml. of concentrated H_2SO_4. Stopper, mix, and let stand near the colorimeter for fifteen minutes at room temperature. Mix again, half fill the colorimeter cup,[1] and read against a standard cholesterol solution made as follows: Pipette 5 mls. of the standard cholesterol solution containing 0.5 mg. cholesterol per 5 mls. of fluid into a cylinder similar to that used above and treat the solution exactly as the unknown was treated. Care must be taken to have both the unknown and the standard exposed to the same kind and intensity of light.

Calculation: The calculation is exactly similar to all foregoing colorimetric calculations. The standard placed at 20:

$$\frac{R_s\ (20)}{R_u\ (n)} = \frac{C_u\ (x)}{C_s\ (0.5\ mg.)}.$$

Solving:

$$x = \frac{10}{n} \text{ mgs. for 20 mls. filtrate, or 1 ml. of whole blood}$$

Or:

$$x = \frac{1000}{n} \text{ mgs. per 100 mls. whole blood.}$$

[1] Unless the cup is provided with a rubber gasket, or otherwise protected from the solvent action of chloroform, it cannot be used. Special fused glass cups are obtainable. The Bausch and Lomb type of cup is desirable. Heavy boiler water-gauge glass tubing is enclosed in a brass cylinder, the bottom of which screws into the cylinder with a tight joint.

Quantitative Determination of Cholesterol by the Method of Leiboff.[1] Principle: (Fig. 256.) A special apparatus is used to extract cholesterol from a known amount of blood which is absorbed on a disc of paper. The cholesterol is then determined colorimetrically. Procedure: Secure an extraction tube (Fig. 256) and add by cylinder 5 mls. of chloroform. Then fit one of the paper discs into the tube.

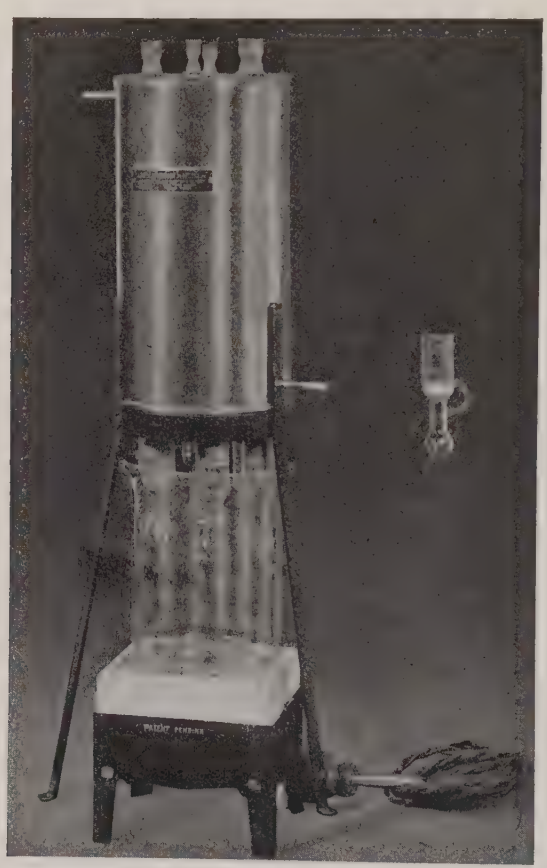


Fig. 256.—Leiboff's apparatus for the determination of cholesterol.

Pipette exactly, by means of your Mohr pipette, 0.25 ml. of whole blood, which should have been collected in contact with oxalate (page 71). Connect the tube with the condenser and submerge the tube in a beaker of boiling water on a water-bath or on an electric heater. Leave half an hour. Then detach the tube, remove the filter-disc, leaving as much of the liquid in the tube as possible.

[1] Leiboff, S. L. (Lebanon Hospital, New York, N. Y.), Journ. Biol. Chem., vol. 61, page 177, 1924.

Immerse the tube in cold water. When cooled to room temperature, add chloroform to the 5 ml. mark on the tube. Preparation of the standard: Place 5 mls. of the standard cholesterol solution[1] in a second tube, add 2 mls. acetic anhydrid and 0.1 ml. (2 drops) concentrated sulphuric acid. Add the anhydrid and acid to the unknown tube. Stopper and mix the contents of the tubes. Leave the tubes.

Fig. 257.—Method of obtaining blood from the superior longitudinal sinus in children too small for venipuncture. The child is six months of age; the shape and size of the anterior fontanel have been outlined; a 5-ml. Record syringe fitted with No. 18 needle is being employed, the needle having been entered for about 6 mms. in the median line at the posterior angle, and perpendicular to the sinus. (Kolmer in Keen's Surgery, vol. viii.)

together, in a beaker of cold water for a half minute in your locker, away from the light. Then compare the colors in the colorimeter. Calculation: $\frac{\text{Reading of the standard}}{\text{Reading of the unknown}} \times 160$ gives milligrams per 100 mls. whole blood.

BLOOD FATTY ACIDS

The residue left after decanting the cholesterol-chloroform solution contains fatty acids derived from 10 mls. of filtrate from 5 mls. of whole blood. Bloor[2] gives methods for quantitative determination of these substances and also lecithins. The details cannot be given

[1] Using pure chloroform, place 100 mls. in a 500 ml. volumetric flask and add exactly 40 mgs. of pure cholesterol. Dissolve and make up to the mark with more chloroform. Each 5 mls. of this solution contain 0.40 mg. cholesterol.

[2] Bloor, W. R., Pelkan, K. F., and Allen, D. M., Jour. Biol. Chem., vol. 52, p. 191, 1922.

here.[1] The fatty acids are in part absorbed by the erythrocytes of the blood and converted into lecithin, in which form they are transported through the blood; in part (especially unsaturated fatty acids, like oleïc) are joined with cholesterol to form esters which travel in the blood-stream. The chief fatty acids are oleïc and linolic, then the saturated acid palmitic, then a small amount of some four double-bond acid (oleïc has but one pair), and finally a small amount of acid with three double-bonds.

In *pathological states* involving an increase in fat in the blood, as in diabetes (lipemia), both plasma and corpuscle show an increase in cholesterol, but the proportion of lecithin to cholesterol is maintained in the same way as in normal blood (in the corpuscle twice as much lecithin as cholesterol); in the plasma equal amounts of lecithin and of cholesterol occur. In diabetes, however, there is a characteristic decrease in lecithin content of the plasma.

The quantity of these various constituents of lipid nature in normal blood is:

	Mgs. per 100 mls. whole blood.
Total fat	600–700
Lecithin	300–350
Cholesterol	140–170

INORGANIC CONSTITUENTS OF THE BLOOD

While the modern indirect methods for the determination of inorganic constituents of blood in minute quantities have been criticized,[2] these methods are in constant use in clinical and research laboratories in the United States and Canada, and in parts of Europe and South America, and elsewhere. The following pages are devoted to such methods as are applicable to medical work, especially for clinical diagnosis. The warning given by Macallum that in careless hands these methods are unreliable must not go unheeded.

Blood Chlorid.—According to Greenwald and Gross,[3] the average normal blood chlorid as NaCl per 100 mls. of whole blood is 0.505 g. Estimated in the plasma, the figures are about 15 per cent. higher (one-sixth higher); that is, about 0.57 g. The chlorion is readily diffusible between corpuscle and plasma (Fig. 42), and this necessitates care in making the determinations. Moreover, the change in CO_2

[1] Directions are given in Folin's Manual.

[2] A. B. Macallum (McGill University, Montreal, Canada), Some Recently Introduced Micro-quantitative Methods and Their Value. Paper presented before the American Society of Biological Chemists, Washington Meeting, December, 1924.

[3] Greenwald, I., and Gross, A., Jour. Biol. Chem., vol. 54, p. 589, 1922.

content of the blood causes a shift in the chlorion;[1] in uncovered vessels the CO_2 escapes, or if plasma is left in contact with corpuscles, CO_2 passes into them, with the result that in either case an increase in plasma chlorion occurs. Sodium does not accompany the migrating chlorion, but remains behind to form NaCl which compensates for the loss of CO_2. Normally, the chlorid varies with the intake of NaCl. During the first forty minutes after a meal there is a fall in blood chlorid; then a return to normal, and from ninety to one hundred and thirty-five minutes, a fall again. On injecting NaCl, about 8 per cent. is retained in the blood and the remainder passes into the tissues.

On a chlorid-free diet high blood chlorids return to the normal figure, which does not fluctuate, even if the supply is further diminished. However, low chlorid diets have been found efficacious in reducing the chlorid content of the body. Urinary chlorid falls on a restricted diet. We have referred to this matter in the preceding Chapter (page 574). In disease the chlorid runs high, in nephritis, cardiorenal conditions, prostatic obstruction, anemia, and in other states in which it is probable that the kidney is the responsible agent. Chlorid is low in any diabetic condition, the chlorid being "washed out" by the excess loss of water; in pneumonia and in febrile conditions; in anaphylactic shock; in emphysema. Loss of water in vomitus likewise causes low chlorid. The concentration of chlorid in blood is a valuable diagnostic agent in certain diseases. The following table shows the characteristic variations in nephritis and in other states (after Gram):[2]

Case.	Condition.	Serum NaCl.	Corpuscle NaCl.	Cell volume.	Whole blood NaCl.	Chlorid concentration.
1	Normal	0.591	0.314	48.0	0.455	Normal or low
2	Normal	0.582	0.321	47.9	0.457	"
3	Normal	0.594	0.330	50.7	0.460	"
4	Normal	0.580	0.336	49.9	0.458	"
5	Polycythemia	0.580	0.307	75.1	0.375	"
6	Edema (physiological)	0.588	0.285	42.4	0.461	"
7	Pernicious anemia	0.588	0.289	17.1	0.538	"
8	Diabetes	0.511	0.276	28.9	0.443	Low
9	Cardiac insufficiency	0.552	0.291	44.0	0.437	"
10	Tuberculosis	0.576	0.313	40.7	0.469	"
11	Pernicious anemia	0.641	0.372	26.0	0.570	High
12	Nephritis	0.620	0.315	44.6	0.484	"
13	Anemia	0.628	0.308	20.0	0.564	"

[1] Page 84 and diagram (Fig. 42).

[2] Gram, H. C. (Girvin Fellow in Research Medicine of the Rockefeller Foundation). Jour. Biol. Chem., vol. 61, p. 337, 1925.

In nephritis involving cardiac inefficiency the highest chlorid content of blood has been found, 0.84 g. per 100 mls. plasma. In such cases edema accompanies the retention of chlorid. Throughout there is a close relation between water and salt metabolism. High blood-pressure has been ascribed to salt retention, and this has been disputed on the basis of blood chlorid, for hypertension does not necessarily occur in high blood chlorid conditions. We have pointed out that the greatest retention is in cardiorenal states in which hypertension does not usually occur. Blood chlorid is held at a normal figure whenever possible. There is an excess of NaCl excretion through the kidneys on salt retention, followed by a decrease with accompanying relief from the high blood-pressure.

It is important to note that a diseased kidney can excrete NaCl during retention of nitrogenous substances.

Following the administration of diuretics, such as theophyllin, a rise in blood chlorids occurs; this is accompanied by an increased excretion of urine (diuresis). From the tissues, NaCl is rapidly passed into the blood. Hence diuresis is intimately connected with the mobilization of chlorid.

Methods for Determining Blood Chlorid.—*Blood Determination.* (Whitehorn, J. Biol. Chem., vol. 45, page 449, 1920.) Pipette 10 mls. of protid free filtrate into a small evaporating dish. Add 5 mls. of standard silver nitrate[1] and stir thoroughly. Add 5 mls. of concentrated HNO_3, mix, and allow to stand 5 minutes. Next add about 0.3 g. powdered ferric ammonium sulphate, and titrate with the standard KCNS solution[2] until a definite pink coloration is obtained which persists for 20 seconds in spite of stirring.

Calculation: 5.00 − titer in mls. = mgs. Cl per ml. of blood.

Cl figure ÷ 0.606 = NaCl.

If figures for plasma are desired use only one-half volume of 10 per cent. tungstate and ⅔ N H_2SO_4 in precipitating protids. Add an extra volume of H_2O to keep the final volume the same.

Van Slyke Method for Larger Amounts of Blood (or Serum).—Principle: The Volhard method (page 790) is employed, and during the precipitation of the chlorids as silver chlorid in nitric acid solution protid is destroyed. Procedure: Pipette 5 gs., or mls., of oxalated blood into a 100-ml. Pyrex Erlenmeyer flask and add 15 mls. of 0.05 normal silver nitrate solution[3] in concentrated HNO_3. Cover with an

[1] 4.791 gms. of $AgNO_3$ in 1 liter H_2O; 1 ml. = 1 mg. Cl.

[2] Made up so that 5 mls. is exactly equivalent to 5 mls. of the silver solution. Dissolve 3 gs. in a liter and titrate against the silver standard, and adjust as indicated.

[3] Appendix.

inverted porcelain crucible top and leave on a water-bath overnight or longer[1] until the supernatant liquid is clear and light yellow. Add 30 mls. of distilled water and about 1.5 gs. of powdered ferric ammonium sulphate violet crystals. Cool to room temperature. Titrate, using 0.05 normal thiocyanate solution. The end point is the same as in the urinary Volhard method.

Calculation: Subtract the correction figure,[2] 0.04 ml., from the burette reading. Then:

$$\text{NaCl per liter blood or plasma} = 0.585\ (15 - \text{mls. CNS}).^{3}$$

Van Slyke Method for Small Amounts of Blood. —Pipette 1.0 ml. of whole blood (or serum) into a 100-ml. Pyrex tube 200 × 20 mms. Add 3 mls. of the silver solution used in the preceding method. Digest as described above. Add 6 mls. of 5 per cent. ferric ammonium sulphate solution and cool the tube to room temperature. Titrate, using 0.02 normal thiocyanate solution. Calculation: After making the correction, as before,

$$\text{Gs. of NaCl per liter whole blood or serum} = \frac{1.17\ (7.5 - \text{mls. of CNS used})}{\text{mls. of whole blood or serum}}.$$

BLOOD PHOSPHORUS

The total phosphorus (inorganic and organically bound) of the blood is of no significance at the present time.[4] The inorganic phosphorus, however, has interest in clinical work and in studies of the normal metabolism of phosphorus and of inorganic substances in general. There seems to be no organic phosphorus in the serum that can be hydrolyzed by acid hydrolysis. This harmonizes with the studies of lecithin[5] which Bloor finds confined in part if not altogether to the corpuscle.

[1] If serum is used the time is greatly shortened. The clear yellow supernatant liquid will appear at the end of the 2.5-hour period.

[2] This represents an excess of thiocyanate necessary to give a clear end-point in the titration.

[3] This is derived from the general expression: NaCl per 1000 mls. whole blood = $\frac{2.925\ (\text{mls. of 0.05 normal } AgNO_3 \text{ used} - \text{mls. 0.05 normal thiocyanate used})}{\text{mls. whole blood (or serum) taken}}$, in which 2.925 is derived from the molecular weight of NaCl (58.5) used in making a normal solution. For an 0.05 normal solution, corresponding to the normality of the $AgNO_3$ and the thiocyanate solutions, we have $0.05 \times 58.5 = 2.925$. Sodium, potassium, or ammonium thiocyanate may be used.

[4] For a method of determining total blood phosphorus see Smith, C. S., and Brown, A. L. (Ohio State University, Columbus), Jour. Lab. and Clin. Med., vol. 9, p. 203, 1923.

[5] For a method of determining the phosphorus derived from lecithin, see Whitehorn, J. C. (Biochemist to the McLean Hospital, Waverly, Mass.), Jour. Biol. Chem., vol. 62, p. 133, 1924.

The blood phosphorus in normal states decreases from infancy to maturity (after Myers):

	Mgs. per cent.
Average normal blood inorganic P in infant	5.05
Average normal blood inorganic P in adult	3.70

In health such figures are retained without marked variation. Phosphorus is concerned in some way with glucid metabolism. On injecting insulin into a normal person the inorganic phosphate of the blood falls. A hexosephosphate may form (page 176).

In disease characteristic variations occur:

(1) Retention due to nephritis. In early stages of interstitial nephritis there is little or no change in blood phosphorus, but in advanced, chronic nephritis there may be such a retention as to cause a phosphate acidosis, referred to previously (page 479). In such instances the inorganic phosphorus may increase over three times normal.

(2) In rickets the inorganic phosphate of the blood is reduced to a greater or less extent. It may reach as low a figure as 2 mgs. per cent. serum. However, it is difficult if not impossible to correlate the blood phosphorus figure with the state of health of the patient. Curative measures (irradiation and the feeding of irradiated foods and of foods such as cod-liver oil) cause a rise of blood phosphorus coincident with a restoration of a calcium-phosphorus ratio, deposition of bone, etc.

(3) The healing of wounds, like the knitting of bone, is frequently accompanied by an increase in blood phosphorus. A decrease to normal ensues when healing has taken place. In some cases of bone fracture the Roentgen ray fails to show when union has occurred, and in such cases the quantitative study of inorganic blood phosphorus, when carefully controlled, is of value in following the course of treatment.[1]

Method for Determining Inorganic Phosphate of the Blood.—*Method of Fiske and Subbarow.*—Into a test-tube pipette 8 mls. of a 10 per cent. trichloracetic solution. Slowly rotate the contents of the tube and while they are in motion, add exactly 2 mls. oxalated blood (2 mgs. potassium oxalate per ml. of blood).[2] Stopper and agitate the contents. Filter through a clean, dry filter. Measure 5 mls. of the filtrate into a 10 ml. volumetric flask. Add 1 ml. special ammonium molybdate solution[3] for blood. Mix. Then add 0.4 ml.

[1] Eddy, W. H., and Heft, H. L., Jour. Biol. Chem., vol. 55, p. xii (Proceedings), 1923.

[2] Whole blood, plasma or serum may be used.

[3] Weigh 2.5 gs. ammonium molybdate and make up to 100 mls. with 3 normal H_2SO_4 solution.

sulphonic acid reagent (Appendix). Dilute to 10 mls. Mix. Preparation of standard: Into a 50 ml. volumetric flask pipette 2.5 mls. of phosphate standard, containing 0.2 mg. phosphorus. Dilute to about 30 mls., add 5 mls. of ammonium molybdate[1] solution and then 2 mls. of special reducing reagent (sulphonic acid reagent; see Appendix). Dilute to the mark, mix and read in the colorimeter after allowing the solutions to stand 5 minutes.

It is to be noted that the ammonium molybdate solution added to the blood filtrate contains 3N H_2SO_4 in order to compensate for the acid added to prepare the filtrate.

Calculation: Setting the standard at 20.0 mms.:

$$\frac{20 \times 4}{\text{reading}} = \text{mgs. per 100 mls. of fluid taken.}^{2}$$

If the amount is less than 2 mgs. per cent., increase the quantity by adding a known amount of phosphorus (a suitable amount is 0.016 mg. or 1.0 ml. of a solution made by diluting 20 mls. of the regular standard to 100 mls. Subtract 1.6 mgs. from the result.

Method of Benedict and Theis.[3]—Principle: The phosphate is precipitated as phosphomolybdate by adding a solution of molybdenum trioxid. The molybdenum is then reduced by means of hydrochinon[4] in a sulphite solution, in a water-bath. The reducing solution is highly colored blue and is compared in a colorimeter with a standard phosphate solution that has been similarly treated.

Procedure: Preparation of the sample: By means of a hypodermic needle withdraw 5 mls. of blood from the subject and inject the blood into a clean 15-ml. centrifuge tube. Permit the clot to form, and while doing so balance the tube against a similar tube, using water. Centrifuge at low speed for five minutes or longer. Avoid hemolysis; specimens showing hemoglobin in serum are to be rejected.

The determination: Pipette 2 mls. of serum into a 10-ml. volumetric flask. Add about 1 ml. of distilled water. Add also 4 mls. of 20 per cent. trichloracetic acid. Mix. Dilute to the mark, 10 mls., with distilled water. Permit the flask to stand for ten minutes or longer, then pour the contents upon a dry, ashless filter in a small funnel. Pipette 5 mls. of the filtrate into a test-tube and add 3 mls.

[1] 2.5 gs. ammonium molybdate are placed in a 100 ml. volumetric flask and diluted to the mark with 5 normal sulphuric acid solution.

[2] A blank should be run and the amount of phosphorus obtained therefrom should be subtracted from this result.

[3] Benedict, S. R., and Theis, R. C. (page 812), Jour. Biol. Chem., vol. 61, p. 63, 1924.

[4] Hydrochinon (*p*-di-hydroxy-benzene), $C_6H_4(OH)_2$, is similarly used in photography to reduce silver salts.

of distilled water. Then add 1 ml. of the special molybdic acid solution, diluted immediately before use with an equal volume of concentrated sulphuric acid. Finally add 1 ml. of the special hydrochinon-sulphite solution. Stopper, invert the tube in order to mix the contents, and leave in a boiling water-bath. The standard: Into a test-tube, similar to that used for the unknown solution, pipette 5 mls. of a solution[1] of potassium hydrogen phosphate, KH_2PO_4, and treat exactly as you have the serum. Place in the boiling water-bath with the tube containing the unknown.

Calculation (standard at 20 mms.):

$$\frac{R_s\ (20)}{R_u\ (n)} = \frac{C_u\ (x)}{C_s\ (0.025\ mg.)}.$$

Solving:

$$x = \frac{50}{n}\ \text{mgs. per 100 mls. serum.}$$

CALCIUM IN BLOOD

In blood chemistry, calcium content is usually expressed as milligrams of calcium per 100 mls. of serum (9.2 to 11.3 mgs. per cent. normal serum). No difference exists between the Ca content of normal serum and plasma. Cameron and Moorehouse[2] find that normally, after clotting, the Ca used in forming thrombin becomes free in the serum. In parathyroidectomy tetany, however, the clot holds the calcium.

Serum calcium varies within narrow limits (normally about 1 mg. per 100 mls. per 24 hrs.). Of the serum Ca, about 40 per cent. is a union of undissociated Ca with protid; this is the so-called "non-diffusible calcium of the blood." Some calcium occurs as diffusible Ca.

Collip has shown that parathyroid hormon obtained by his method may quadruple the content of calcium in the blood. However, other internal secretions (insulin, pituitary hormon) likewise have the same ability but to a smaller extent. When insulin and pituitary hormone are mixed, the calcium augmenting effect is suppressed.

Calcium varies in a characteristic manner in certain physiological and pathological states. Diffusible Ca is more constant than non-diffusible calcium. After clotting, an increase in diffusible Ca occurs. Two factors operate to maintain normal serum calcium physiolog-

[1] Page 892.

[2] Cameron, A. T., and Moorehouse, V. H. K. (page 24), Journ. Biol. Chem. vol. 63, p. 687, 1927.

ically; (1) The parathyroid apparatus and (2) supply of calcium. In pregnancy there is at all times a deficiency, or tendency towards deficiency in this element due to the combined demands of child and mother. Mason[1] showed that ingestion of calcium lactate and chlorid affected the calcium content of blood (plasma Ca) but little (lactate, +1.4–2.6 mgs. per cent.; chlorid, +1.2–4.4 mgs. per cent.). Calcium is higher in youth.

Pathologically, calcium is low following parathyroidectomy, in parenchymatous nephritis and in rickets and, according to Howland, only in these cases. It is deficient in infantile tetany, in epilepsy and sprue; in the tropical disease just mentioned, it is quite low.

Gasometric Method for Blood Calcium of Van Slyke and Sendroy.[2]—Principle: Calcium is precipitated as calcium oxalate, collected by centrifugalization and the oxalate decomposed into oxalic acid which is oxidized by permanganate to CO_2; the CO_2 is then determined gasometrically in the apparatus of Van Slyke and Neill (Fig. 390). Procedure:[3] Into a selected 15 ml. centrifuge tube[4] pipette exactly 2 mls. of clear serum, 2 mls. distilled water and 1 ml. of ammonium oxalate solution 4 per cent. Mix by means of an attenuated, ball-pointed stirring-rod. Remove the rod and wash with one drop of water. Let stand at least 30 minutes and then centrifuge for 5 minutes. Decant the supernatant liquid carefully and while the tube is in an inverted position, stand it on a piece of filter-paper on your test-tube rack. Leave 5 minutes. Then invert the tube, add 2 mls. of normal H_2SO_4 solution and, by means of the stirring rod, cause the whole of the precipitate to go into solution. Pour the solution quantitatively into the burette of the gasometer, using an amount of water for washing such that the volume in the burette will be 6 mls. Bring the solution into the mixing chamber below cock b by proper manipulation of the cocks b and e. Evacuate the chamber and shake for 2 mins. expelling the dissolved air and CO_2. Now add 1 ml. decinormal permanganate.[5] Evacuate the chamber and shake for 3 mins. Make the volume 1.5 ml. and read the manometer as p_1. Now add 1 ml. of 5 normal NaOH, make the volume again 1.5 ml.

[1] Mason, E. H. (Royal Victoria Hospital, Montreal), Journ. Biol. Chem., vol. 47, page 3, 1921.

[2] For the privilege of reproducing this method the author is grateful to Dr. Van Slyke.

[3] The earlier part of this method is that of Tisdall and Kramer; see page 864.

[4] The tube must be acutely pointed in order to hold the impacted precipitate.

[5] In order to insure against any undecomposed carbonate, the permanganate solution is previously treated with 5 mls. of decinormal sulphuric acid solution for each 100 mls. of solution.

and read the manometer as p_2. A control must be run at some time with the solutions, the quantity c being $(p_1 - p_2)$ for this test. Calculation:

$$p_1 - p_2 - c$$

is calculated and the following table is entered with the pressure thus calculated in order to obtain the milligrams of Ca per 100 mls. serum:

Temperature.	Factor.	Temp.	Factor.
15	0.0673	25	0.0628
16	667	26	623
17	661	27	619
18	658	28	615
19	652	29	611
20	0.0649	30	0.0608
21	645	31	605
22	639	32	600
23	636	33	598
24	632	34	595

SODIUM IN BLOOD

In acid-base studies it is necessary to determine the sodium content of the blood. Sodium is a characteristic constituent of the plasma and not of the corpuscles. The average normal amount of sodium is 324 mgs. per 100 mls. net weight blood-serum.[1] For whole blood sodium runs 200 mgs. per 100 mls. Sodium plays a rôle in the maintenance of neutrality in the body (page 84). Clinically, sodium is not correlated with conspicuous variations in body states.

Method of Kramer and Gittleman.[2]—Principle: Sodium is precipitated from the blood-serum as sodium pyro-antimoniate, $Na_2H_2Sb_2O_7$. The antimony is then determined on the precipitate after removal of all excess antimony by means of centrifuging, and after dissolving the precipitate in concentrated hydrochloric acid. For the determination the antimony is reduced and iodin is at the same time released from potassium iodid that has been added. Finally, the iodin is determined by means of thiosulphate.

Procedure: Pipette 2 mls. of serum into a 50-ml. centrifuge tube. Add 10 mls. of the special pyro-antimoniate solution[3] and 3 mls. specially prepared ethanol 95 per cent.,[4] drop by drop, while agitating the contents by means of a small "policeman." Wash off the rod

[1] From the data given by Kramer, B., and Gittleman, I. (Johns Hopkins University, Baltimore, Md.), Jour. Biol. Chem., vol. 62, p. 353, 1924.

[2] Page 863.

[3] Appendix.

[4] Redistilled over sodium.

with 2 drops of water, stopper the tube, and let it stand for half an hour. Remove the stopper and centrifuge at moderate speed for five minutes. Using a small 1-ml. pipette fitted with rubber bulb withdraw all but about 2 mls. of the supernatant fluid. Add 10 mls. of 30 per cent. ethanol solution, mix, and centrifuge again for one minute. Remove all the supernatant fluid that is possible.

Determination of the antimony in the precipitate: To the precipitate add 5 mls. of concentrated hydrochloric acid and stir with the "policeman." Pour quantitatively into a 250-ml. beaker, using not over 10 mls. distilled water for washing the residue into the beaker. Fill a burette[1] with decinormal sodium thiosulphate solution and adjust the meniscus. To the beaker add 2 mls. of 20 per cent. potassium iodid solution. Add the thiosulphate rapidly until the brown color produced when the iodid was introduced disappears, and then add 0.5 ml. of starch indicator. Continue the titration until the solution becomes clear like water.

Calculation: Each ml. of the thiosulphate used from the burette corresponds to 1.15 mgs. of sodium.

Total Titrable Base in the Blood.—Method of Sumner and Hubbard: This method has been given on page 801.

Iron in Blood, Quantitative Method of Smirk.[2]—Principle: Blood protids are oxidized by ammonium persulphate and nitric acid. The iron left after this process is then determined as thiocyanate colorimetrically, the standard being composed of certain dyes in acid solution. Following the suggestion of Marriott and Wolf,[3] aceton is used to intensify the color. Reagents: Powdered ammonium persulphate; concentrated nitric acid; aceton; ammonium thiocyanate solution 24 gs. per 100 mls. dist. H_2O. Standard: Cochineal solution, 4 mls. of a solution made by crushing 10 gs. of the dye in 100 mls. of 25 per cent. ethanol; 3.2 mls. of a solution made by dissolving 1 g. dye in 500 mls. of a 50 per cent. ethanol solution; 4 mls. of 1:5 HCl solution. After mixing, dilute with a solution of equal parts of aceton and H_2O until the quality of the color is indistinguishable from that of the thiocyanate solution. Standardize the artificial against a standard made by dissolving 0.70 g. pure ferrous ammonium sulphate in about 50 mls. of water in a liter flask, add 20 mls. 10 per cent. Fe-free H_2SO_4 and a few drops of 0.1 n. potassium permanganate solution. Dilute

[1] A 15-ml. Folin-Wu blood sugar pipette will serve (Fig. 237).

[2] Smirk, F. H. (Manchester Royal Infirmary, England), Biochem. Journ., vol. 21, page 36, 1927. See: Fowweather, F. S., idem, vol. 20, p. 93, 1926.

[3] Marriott, W. McK., and Wolf, C. G. L., J. Biol. Chem., vol. 1, page 456, 1906.

to the mark. Each ml. contains 0.1 mg. iron. Procedure: Two tenths of a ml. of blood are placed in a Pyrex test-tube. Wash the pipette with a few drops of distilled water. Add 0.33 g. ammonium persulphate, followed by 2 mls. conc. HNO_3. Heat over a microburner until the solution becomes clear. Heat one minute longer; after the solution has passed through a darker, then lighter stage, cease heating. Now add 6 mls. of H_2O. Transfer to a 50 ml. volumetric flask, rinsing the test-tube with small portions of water. Complete the washing by adding to the test-tube 25 mls. of aceton and then transfering the contents to the flask. Place the tube in a beaker of cold water for 5 minutes. Then add 5 mls. of the ammonium thiocyanate solution and make up to the mark with water. Compare in a colorimeter with the standard made as described on page 865.

SUMMARY OF CHAPTER XVI

1. Quantitative methods have been given for determining from small amounts of blood the various constituents which are important in metabolism.

2. These methods are, as a rule, colorimetric or volumetric.

3. Blood constituents vary according to endogenous and exogenous conditions.

Endogenous variations may be physiological ("normal"), due to a change in metabolism; or they may be pathological, as in goiter, diabetes mellitus, and nephritis.

Exogenous variations are those concerned with the food. When, for example, less nitrogenous food is ingested, the non-protid nitrogen of the blood is lowered.

4. In general, the constituents of the blood vary within very narrow limits. The substances in the tissues and those in the excretions (urine) may vary conspicuously, but the blood, which is simply a transporting system, does not change except in serious pathological conditions.

5. The blood is a diagnostic agent of great importance. Certain diseases cannot be accurately diagnosed without blood analysis. The modern treatment of diabetes mellitus by diet and insulin requires immediate knowledge of the blood-sugar content. The various forms of nephritis are best followed by blood chemistry.

6. The knowledge of the intermediate metabolism of substances, and of metabolism in general, has been greatly enhanced by modern blood chemistry.

7. Three great divisions of blood chemistry exist:
   (*a*) Nitrogenous.
   (*b*) Inorganic.
   (*c*) Reaction of medium, acid-base relations.

Each has its clinical application.

SUGGESTED READINGS

Many references have already been given as footnotes in the Chapter. The rapid progress of blood chemical methods and results makes it impossible to follow the subject without resort to *periodicals*. The following sources of modern methods should be used to keep abreast of the subject:

Biochemical Journal, University of Chicago Press, Chicago, Ill. Price, annually, $14.00.

Journal of Biological Chemistry, Rockefeller Institute, 66th St. and Avenue A, New York, N. Y. About $20.00 annually (4 volumes at $5.00 each).

Proceedings of the Society for Experimental Biology and Medicine. Obtainable from the Secretary, College of the City of New York, N. Y. Price per year, $5.00.

Journal of the American Medical Association. Obtainable from the Association, Chicago, Ill. Per year, $5.00.

Journal of Clinical Investigation. Obtainable from the publishers, Williams & Wilkins, Baltimore, Md. Per year, $5.00.

Journal of Laboratory and Clinical Medicine, obtainable from the publishers, C. V. Mosby Co., St. Louis, Mo. Per year, $6.00.

Many of the *books* bearing upon blood chemistry are included in the list given at the end of the last chapter (Chapter XV, page 804). In addition, and with especial reference to blood chemistry, the following are valuable reference books:

Beaumont, A. E., and Dodds, E. C.: Recent Advances in Medicine, Clinical, Laboratory, Therapeutic, Philadelphia, P. Blakiston's Son & Co., 1925.

Folin, O.: Laboratory Manual of Biological Chemistry, New York, D. Appleton & Co., 1925.

Myers, V. C.: Practical Chemical Analysis of Blood, St. Louis, C. V. Mosby Co., 1924.

Rockwood, E. W.: A Laboratory Manual of Physiological Chemistry, Philadelphia, The F. A. Davis Co., 5th ed., 1924.

Underhill, F. P.: A Manual of Selected Methods in Biological Chemistry, New York, John Wiley & Son, 1923.

Hawk's Physiological Chemistry (cited on page 26) contains many of the older methods and most of the newer methods of blood analysis.

CHAPTER XVII

METABOLIC STUDIES ON URINE AND BLOOD

"Nobis serimus, nobis metamus."[1]

In the foregoing pages methods have been outlined for the analysis of urine and blood. Since the diagnosis of many diseases depends upon the application of such methods, they are important clinically. Our knowledge of the normal and abnormal metabolism of animals has been gained largely through the analysis of blood and urine. It is for these reasons that an intensive study of urine and blood analysis is included in biochemistry.

Whenever possible the studies about to be undertaken should be conducted on the student himself, or his co-workers, thus affording him concrete examples of normal or abnormal findings. Also pathological blood and urine specimens should be available for comparative studies. These are always obtainable from the hospitals, or from practising physicians, but are not always available at such a time as to fit into a particular scheme of presentation, such as that given here. They must, therefore, be studied whenever they can be obtained. In some cases it is possible to imitate actual pathological specimens by introducing such substances as protid, glucose, or aceton into normal specimens. However, natural specimens are preferable, as artificially made solutions do not give identical reactions. For example, when glucose is added artificially to normal urine the colloidal relations are different from those of diabetic urine, and reduction of copper in a Fehling or Benedict qualitative test is also different from that of an actual specimen. If an artificial solution, after copper reduction, be viewed by reflected light, the color is deep brown or red; when viewed by transmitted light, as when the light passes through the solution directly to the eye, the color is bluish-green unless the glucose is added to high concentration. This effect is especially evident if pure glucose is used in aqueous solution. The colors obtained by copper reduction methods depend upon the degree of dispersion of the copper substances, and the dispersion depends, in turn, upon the constitution of the solvent.

[1] What we sow, we reap.

Preparation for the Exercises in This Chapter.— For the collection of urine each student must provide himself with two 1-liter prescription bottles, or two 500-ml. bottles, graduated in milliliters. These are obtainable at any pharmacy. He will also need 100 mls. of toluene to be used as a preservative of the urine. Five mls. are added to each liter of urine and then the specimen thoroughly shaken. Unless otherwise directed, all twenty-four-hour specimens must be brought to class. A twenty-four-hour collection is made as follows: At 8.00 A. M. empty the bladder and reject the specimen. Thereafter until 8.00 the following morning save all urine voided. At the beginning of the collection add about 3 mls. of toluene to each 500-ml. bottle. Use 5 mls. for a liter bottle, whether the urine is expected to fill it or not. Under certain circumstances departures from this method of collecting the urine will be indicated. Care must be exercised to include in the collection all precipitates that may form. The following data must be determined upon each specimen unless otherwise directed:

1. Total volume.
2. Specific gravity.
3. Color.
4. Precipitate, suspension, sediment.
5. Reaction to litmus paper.

A variation in diet or water intake or change of body condition (infection, etc.) during the period for which the determination is made should be recorded in your note-book.

PRELIMINARY STUDIES ON URINE

EXERCISE 1.—Determine the total solids in the urine according to the methods of Long (page 694). To 100 mls. of urine add 1 g. of sodium chlorid and to a second 100-ml. specimen add 1 g. of glucose. Determine the total solids in each case. Save each specimen for the following Exercise.

EXERCISE 2.—Using the mixed urine of Exercise 1, precipitate the uric acid according to the method given on page 721. During the precipitation study the chemistry and reactions of uric acid as follows:

(*a*) Use the crystal uric acid obtained at the store-room.[1] Test the solubility in cold and hot water; in ethanol and in 1 : 10 HCl

[1] This uric acid has been collected from the precipitations made by previous classes and saved.

and 1:10 solution of 10 per cent. NaOH (0.01 per cent. NaOH). Test the solubility in 1 per cent. sodium carbonate solution.

(*b*) Using crystals from the store-room, perform the murexid test (page 721) and the Folin qualitative test (page 721). When the crystals have been obtained from the precipitate, repeat the tests under (*a*). Study the crystals under the microscope. Make drawings of typical crystals.

Deliver to the store-room any uric acid not utilized in these Exercises.

Exercise 3.—Quantitative uric acid determination: Determine the concentration of uric acid in a twenty-four-hour specimen, using the method given on page 726. Calculate the uric acid nitrogen in this specimen and enter the result in your note-book.

Exercise 4.—Secure a sample of urinary calculi from the laboratory and, using the scheme of analysis given on page 724, record the results of the examination.

Exercise 5.—Secure from the store-room urea crystals. Examine the crystals, making a drawing of the typical form. Draw also the forms of urea nitrate crystals (page 702). Test the solubility of the crystals in:

(1) Cold and hot distilled water.
(2) Ethanol, 50 and 95 per cent.
(3) Acetic acid, glacial and 50 per cent.

Exercise 6.—Determine, quantitatively, the urea in a twenty-four-hour quantity of urine, using the method given on page 710. Express the result as urea nitrogen.

Exercise 7.—Determine the urea on the same specimen by the method given on page 712.

Exercise 8.—Determine the amount of ammonia in the urine that was used for the two preceding exercises. Express as ammonia nitrogen.

Exercise 9.—Apply the Jaffé test for creatinin to 5 mls. of a mixed specimen of twenty-four-hour urine. Into each of two test-tubes place 5 mls. of urine. Into tube (*b*) pour 1 ml. of a 1 per cent. solution of glucose. Apply the Jaffé test, using the same amounts of reagents for each tube. Refer to page 163 for the picric acid reaction for glucose and to page 739 for the picric acid test for creatinin (Jaffé)

Exercise 10.—Determine, quantitatively, the creatinin in a twenty-four-hour sample of urine (page 738). Compare your results with those of other members of the class and determine whether there is any correlation between creatinin and individual bodily characteristics by the following method: Plot, on coördinate paper, as abscissæ,[1] the weights of the subjects, and as ordinates, the corresponding creatinin excretion. If there is a correlation, the two factors will vary in the same way.[2] The creatinin excretion figure will be utilized later (page 872, 14).

Exercise 11.—The urine of a child has been provided. Determine the content of creatin according to the method given on page 737. This Exercise may be performed using urine to which a known quantity of creatin, obtained from beef-extract, has been added.

Exercise 12.—Determine the total nitrogen in 5 mls. of a mixed twenty-four-hour specimen of urine by each of the following methods:[3]

1. The Kjeldahl method, pages 287 and 697.
2. The Folin method, page 697.
3. The micro-method, page 698.

Exercise 13.—Repeat the above procedure, using urine from a patient with Bright's disease. In this case the protid must be separated from the non-protid substances of the urine. Acidulate 50 mls. of the urine with 2 mls. of concentrated desk reagent hydrochloric acid, mix, and boil until the albumin has coagulated. Filter, cool the filtrate, and apply the biuret test. If negative, proceed; if positive, add a knife-point of kieselguhr, mix, and filter through a dry filter. Or 5 mls. of 5 per cent. trichloracetic acid solution may be

[1] A term used by René Descartes, French mathematician, founder of analytical geometry, to designate the horizontal base line (Latin *ab*, from, and *scindo*, to cut; that is, to remove) or axis in a system of coördinates The ordinate (Latin *ordinata applicata*, the line applied parallel to the vertical axis) is the vertical axis. With these two lines fixed and the points of intersection known, any relation between two factors or sets of factors, such as body weight and creatinin excretion, can be definitely expressed. The system is exactly like that used when we say "The College of Physicians is at 59th Street and Tenth Avenue." For practical directions concerning statistical methods see Pearl, R. (Johns Hopkins School of Hygiene and Public Health), Introduction to Medical Biometry and Statistics, Philadelphia, W. B. Saunders Co., 1923

[2] Two quantities may vary in a linear way (lines parallel), inversely (lines standing at an angle to one another), etc.

[3] Instead of each student performing each Exercise, one group of students may determine total nitrogen by the Kjeldahl method, another by Folin's method, and a third by the micro-method.

added to the original urine. Let the specimen stand for five minutes, then filter. Use either of these filtrates as a basis for the determination of non-protid nitrogen ("total nitrogen"). For comparison determine, by the Esbach or Quick method (pages 766 and 765), the total protid of the same urine, and, using the factor 6.25, determine the nitrogen contained in that amount of protid. Compare this amount of nitrogen with the total nitrogen (N. P. N.) of the urine determined first in this Exercise.

Exercise 14.—Total the amounts of nitrogen from each of the constituents sought in Exercises 3, 6, 8, and 10. Subtract this from the total nitrogen figure. What substances make up the remainder of the nitrogen?

Exercise 15.—By means of the modified Volhard method (page 788) determine the concentration of chlorids in a twenty-four-hour sample of urine.

Exercise 16.—By means of the method given on page 792 determine the inorganic phosphates in the same sample of urine.

Exercise 17.—Using the same sample of urine, let one group of students determine the total sulphates; another, the inorganic; and a third, the ethereal and neutral sulphur. Place the results upon the blackboard for comparison. Repeat, using as many twenty-four-hour specimens of urine as possible.

Exercise 18.—Preparation of specimens for future Exercises: In order that material be available for future studies in metabolism certain preparations should be begun at this point:

(*a*) *Preparation of creatinin for standards* in creatin and creatinin determinations. Follow the procedure given in the Appendix. Creatinin may be left as zinc chlorid, or it may be purified to creatinin. The collection of urine may be made from classes other than the one taking biochemistry or from preserved, discarded urine from the biochemical laboratory. Fresh urine, however, is always preferable. By pooling specimens from a group of students enough urine can be obtained (say 10 liters) to afford considerable creatinin. The standard creatinin solution should be prepared during intervals in the following Exercises.

(*b*) *Preparation of Standard Nitrogen Solution.*— Secure from the store-room a weighing bottle of purified[1] ammonium sulphate crystals, receiving the bottle on a piece of filter-paper in order to avoid soiling the glass with oil from your hand, as this would increase the weight of the bottle. Place the bottle on the left-hand pan of an analytical balance and on the right pan place weights to balance the bottle to the fourth decimal place (tenths of milligram). Now open the bottle with crucible tongs with rubber tips and remove with a knife-point or cardboard scoop as many crystals as will lie on a 5-cent piece. Restopper the bottle and transfer the crystals to a 100-ml. beaker. Weigh the bottle again. By subtraction find the weight of the crystals removed. Then, by proportion, determine what volume of water must be added to them to give 1 mg. of nitrogen per 10 mls. of solution. The proportion is 0.4716 g. per liter solution. Then, if the weight is 0.3200,

$$\frac{0.4716}{0.3200} = \frac{1000}{x}; \; x = 678 \text{ mls.}$$

Dilute the crystals with about 50 mls. of distilled water and transfer them quantitatively to a liter flask, using about 500 mls. of water. Into a 500-ml. volumetric flask[2] pour the solution to the mark and transfer the residue to a 100-ml. volumetric flask; dilute to the mark. You have now 600 mls. of solution, and 678 mls. are required. Pour the contents of the two volumetric flasks into the original flask, and, using the 25-ml. volumetric pipette, add three quantities of 25 mls. each of distilled water, and then with the 1-ml. Ostwald-Folin pipette add 3 mls. more. The flask now contains exactly 678 mls. of solution, each 10 mls. of which contains a milligram of nitrogen. Add 5 mls. of toluene to the solution, stopper, mix thoroughly, and let stand for use in later Exercises. It is well to determine the nitrogen in this solution by making a Kjeldahl determination (page 697), or a modification of this method for total nitrogen; or by checking a nesslerized specimen against the laboratory standard.

Exercise 19.—Make an analysis of unknown urine given you, the nitrogen content of which is known to the instructor:

1. Total nitrogen. Let one group of students perform the analysis by the Kjeldahl method (page 697) and another by the micro-method (page 698).

[1] The method of purifying ammonium sulphate for the making of standard nitrogen solutions is given in the Appendix.

[2] Any combination of flasks may be used, but the fewer the transfers, the more accurate the work.

2. Ammonia. Let one group make the determination by the Permutit method (page 715), and another by the Malfatti method (page 718).

3. Urea. Let one portion of the class use the direct nesslerization method (page 710), and another the Marshall method (page 712).

4. Uric acid. Let one section use the centrifuge method (page 726) and another the direct method (page 725).

Continue respective groups, making determinations for:

1. Creatinin (page 738).
2. Chlorid (page 788).
3. Inorganic sulphate (page 795).
4. Phosphate (page 796).

When all the determinations have been made, place the results on the board and compare them. In case of discrepancies the student whose figures vary from those of others using the same method must repeat his determination.

Exercise 20.—Using a sample of a twenty-four-hour specimen of your own urine, determine the total nitrogen by the micro method (page 698) and also the sulphate and sulphur (page 796). Compare the results you have obtained with those of other students, paying especial attention to the ratio $\frac{\text{neutral sulphur}}{\text{total nitrogen}}$. Is there any correlation? Plot your results against those of other students at your desk.

Exercise 21.—By means of the method given on page 772 determine the "normal" glucid content of a specimen of urine. Using a part of the same specimen, perform a fermentation test and note the presence or absence of fermenting sugar. Retain the liquid left in the fermentation tube and analyze for reducing substance again by the first method used.

Exercise 22.—Preparation for this Exercise: In place of collecting the full twenty-four-hour quantity of urine on this day, save only that excreted from 7.00 to 10.00 A. M., after rejecting the night urine at 7.00 A. M. Using this sample of three-hour urine, make the following determinations:

1. Total nitrogen (page 697), Folin's method.
2. Urea nitrogen (page 710).
3. Creatinin (page 738).
4. Ammonia nitrogen (page 710).

Compare the results with those given in Folin's Manual (cited on page 26[1]). If time permit, uric acid and the inorganic substances may be determined also.

EXERCISE 23.—Preparation for this Exercise: The class is divided into four groups or other convenient units:

1. This group refrains as much as possible from drinking all liquids and from taking liquid in the form of soup, etc., for twenty-four hours. The diet should consist of toast and butter; baked potatoes and butter; griddle-cakes; cottage cheese. If the thirst that follows such an experiment is pronounced, an orange may be used, but only a small amount of liquid should be taken.

2. This group uses liquids freely, especially tea, coffee, cocoa, and other liquids containing diuretics, like caffein.

3. This group maintains a high protid diet (steak, eggs, beans, peas, bread, etc.).

4. This group uses a low protid diet (melons, cream, salads, tea, and other beverages), ice-cream, apples, apricots and other fruits, asparagus, bananas, creamed carrots, beets, cabbage, cauliflower, grapes, onions, oysters, clear soups or vegetable creamed soups, pineapples, white potatoes, prunes, raisins, spinach, and white rice).

Study urines collected by each individual for the following points:

1. Total volume, color, specific gravity, and total solids.

2. Reaction (quantitative, colorimetric) as follows: Introduce 5 mls. of filtered urine into a Pyrex test-tube similar to those used in Exercise 4, page 71. Add 1 drop of the indicator methyl-red (Appendix) and compare with the standards which were made (page 66). If the colors fail to match, repeat with a new preparation, using brom-cresol-purple or brom-thymol-blue.

3. Total nitrogen, urea nitrogen, ammonia nitrogen, creatinin, and uric acid.

Compare the results and note any correlations with the diet.

EXERCISE 24.—High and low chlorin diets. The class is divided into two groups:

A. Low chlorin diet (see Strouse and Perry)[2] as follows:

[1] This chart should be copied on the blackboard for student reference.

[2] Strouse, S., and Perry, M. A. (Michael Reese Hospital, Chicago), Food for the Sick, Philadelphia, W. B. Saunders Company, 1917 (out of print, but obtainable in libraries). Also Friedenwald, J., and Ruhräh, J. (University of Maryland, Baltimore, Md.), Diet in Health and Disease, Philadelphia, W. B. Saunders Co., 1925.

Total salt, about 7 gs.:

Breakfast: Rice, boiled, buttered with salt-free butter, the so-called "fresh" butter of the market.
Tea, coffee, or cocoa with cream.
Egg-nog (2 eggs beaten into 250 mls. of whole milk).

Lunch: Two lamb chops, or small steak, fried or broiled, salt free; olive oil or cotton-seed oil may be used in preparing this dish.
Toast, preferably of salt-free bread, like the Jewish unleavened bread, and with salt-free butter.
White potatoes boiled in salt-free water. Butter salt free.
Gelatin pudding, baked apple, apple sauce, etc.

Dinner: Meat as for lunch, potatoes sauté, creamed or French fried in oil, carrots, beets, etc., boiled.
Salads (banana, lettuce and tomato, etc.), with French dressing (oil and vinegar, no salt, but with pepper if desired; or salt-free mayonnaise).
Dessert of custard, made salt free, stewed fruit, toast and cottage cheese (cream cheese), etc.

B. This group maintains a high salt diet somewhat as follows:

Total salt, over 15 gs.:

Breakfast: Bacon and eggs (salted); potato chips.
Oatmeal, salted to taste.
Beverage.

Lunch: Soup made from meat extract ("Steero-cubes," etc.) salted as highly as possible to taste. Saltine crackers.
Ham, fried or broiled.
Bread, brown ham gravy.
Hashed brown potatoes, salted.
Dessert: Ice-cream.

Dinner: Soup, like Campbell's[1] vegetable soup, highly salted. Saltine crackers.
Roast beef, salted.
Vegetables, like cabbage, etc., highly salted.
Salad, like bacon and lettuce salad.
Ice-cream, pie, etc.

Determine the chlorid content of the two groups of urines by the method given on page 788. Compare the results. Note the volume, reaction, and specific gravity of the two sets of urines.

B. ADVANCED METABOLIC STUDIES

In several institutions a course in chemical pathology, nutrition, etc., is included in a course in biochemistry, either during the same or a subsequent year. The following Exercises are designed to be offered in such a course. In many instances the Exercises may be carried along with those given above, either as additional studies of the nature of problems or as demonstrations.

[1] Dr. Bartlett, of the Campbell Soup Company, Camden, N. J., reports the average sodium chlorid content of the Campbell soups, diluted for use, as about 1 g. per 100 mls. soup.

EXERCISE 25.—Using the standard nitrogen solution prepared on page 873,[1] analyze the specimen of blood given you according to the method given on pages 814 and 815 for the following constituents:

1. Non-protid nitrogen.
2. Urea nitrogen.

The method for preparing the blood for analysis is given on page 814.

EXERCISE 26.—Using the standard creatinin solution prepared from the creatinin or the creatinin zinc chlorid in Exercise 18(*a*), page 872, determine the creatinin in the blood given you, using the directions for such determinations given on page 835.

EXERCISE 27.—By the method given on page 858, first method, determine the blood chlorid content of the blood filtrate given you.

EXERCISE 28.—By the method given on page 843 determine the sugar content of the blood filtrate given you.

EXERCISE 29.—Pathological specimens: Using the blood filtrate given you, that has been obtained by venipuncture of pathological cases, analyze for the following quantities:

1. Non-protid nitrogen.
2. Urea nitrogen.
3. Uric acid (page 830).
4. Sugar.

Indicate which constituent you find abnormal in quantity.

EXERCISE 30.—The instructor will call for volunteers[2] from whom blood may be withdrawn by venipuncture. In this Exercise the instructor or assistants[3] will withdraw the blood. Chemists appointed from the class will prepare the anticoagulant, receive the blood, and analyze it for the nitrogen, chlorid, and sugar. Place the results upon the blackboard.

[1] In case the work is not continuous the preparation of the standard may be deferred to the present Exercise; or, if the standard has been prepared for some time, it must be checked against a known standard, the value of which is accurately known.

[2] The call should be issued at the meeting preceding the one at which the blood is to be used. The subjects must present themselves for venipuncture before taking breakfast, or the equivalent.

[3] It is always possible to obtain the services of a physician to aid in this class work. It is unwise at this time to permit students to operate unless under strict supervision. Later it is most desirable that the student learn the technic. This may be done on an experimental animal (as explained), but preferably on man.

EXERCISE 31. Preparatory to future work: Later, studies will be made upon deficiencies in diet on the lower animals. In order to prepare such demonstrations the following procedure is recommended:[1]

1. *Vitamin Deficiency.*—A. *Vitamin A.*—Place two white rats in Cage A and two in Cage B. Note sex and age; the laboratory records give these data. Mark Cage A with white paint and clip the ear of each rat with one "V" to indicate that it belongs to this cage; mark "V" in pencil on the label of this cage. Mark cage B with red paint and add a small marking of red paint to the coat of both rats in this cage.

Cage A, Experiment.

Cage B, Control.

Enter into a note-book the day on which the experiment began and other desirable data. Feed both sets of rats the following diet:

Casein, purified, vitamin free[2]	50 gs.
Potato starch, commercial	150 "
Pure leaf lard	40 "
Salt mixture[3]	10 "

In addition, feed to the animals in Cage B fresh butter, 30 gs. The amount of food must depend upon the age and condition of the rats, which should be weighed at frequent intervals (daily, if possible) and their weight curve drawn. The weight should not fall below that at the beginning of the experiment in either case for at least two weeks.[4]

[1] The instructor should appoint small groups of students to arrange these demonstrations. Each group should appoint a member to act as caretaker for the animals, rotating the work as desired.

[2] Page 110, note 1.

[3] This is a mixture of different salts, acids, and bases. The laboratory maintains a stock supply of such a mixture, the ingredients of which are as follows:

Dairy salt[1]	75 gs.
Oyster shell, ground	10 "
Sodium carbonate	2 "
Syrupus ferri iodidi, U. S. Phar. IX	3 mls.
Bone ash[2]	10 gs.

[1] Obtainable from the A. H. Thomas Co., Philadelphia, Pa.

[2] See Wisconsin Agr. Exp. Bull. No. 74.

[4] For normal curves of growth and standard data concerning the white rat see The Rat, Memoir No. 6, Wistar Institute, Philadelphia, The Wistar Institute of Anatomy and Biology, 36th Street and Woodland Avenue, 1924. For the care of the rat as an experimental animal see Breeding and Care of the Albino Rat for Research Purposes, by M. J. Greenman, M. D., and F. Louise Duhring, Philadelphia, The Wistar Institute of Anatomy and Biology, 1923.

At about this time signs of vitamin A deficiency should appear in the experimental animals in Cage A. Note especially the condition of the eyes. Rickets will not appear within the time limit of this Exercise, especially if adult rats are used. Attention will be given later to this condition.

B. *Vitamin B Deficiency.*—Place two pigeons in separate cages. Provide the cages with water, bits of egg or oyster-shell, and feed a plentiful supply of white, polished rice.

To B (control) feed also a mixture of cracked yellow corn, hempseed, barley, and some fresh vegetable like cabbage.

The cage must be kept free from droppings and the water dishes must be boiled at frequent intervals. After about ten days the birds will show the onset of avian beriberi, of which polyneuritis is one of the first symptoms, which becomes acute later, the time varying with the specimens.[1] After the pigeon has become evidently affected (compare Figs. 168 and 196), feed a diet consisting of a mixture of fresh yeast (compressed), hard-boiled eggs, wheat-germ, and cereal grains (cracked whole wheat, etc.). Note the rapid recovery.

C. *Vitamin C Deficiency.*—Scurvy. Guinea-pigs are best for this purpose. Place two male guinea-pigs, characteristically marked, in each of two cages. Feed to both the following diet:

	Parts.
Rolled oats	69
Alfalfa meal	25
Casein	5
Sodium chlorid	1

This diet will produce scurvy in guinea-pigs within two weeks under ordinary conditions (Steenbock's diet, personally communicated, for the favor the author's thanks being extended).

It also usually induces rachitic symptoms in guinea-pigs within three weeks, and unless treated the animals die within about one month. After pronounced scurvy symptoms have developed[2]

[1] In the case of two pigeons that the author had used in the laboratories of Jefferson Medical College for over two years, one had not shown polyneuritic symptoms within the four-week interval that was sufficient to cause that condition in the second bird.

[2] These symptoms are bleeding from the gums, looseness of the teeth, etc., but for the purposes of superficial examination from day to day one may depend upon the assumption by the pig of what is known as the "face-ache" position, that is, applying one cheek to the bottom of the cage. When moving about the affected pig frequently retracts one hind leg as if the soles were tender. It is desirable to kill one pig and have the pathological department section the head of one femur after decalcification.

administer to one set of the pigs (experiment) the following in addition to the diet just mentioned:

Orange juice	5 mls. (in milk)
Carrots, preferably small young, that have stood in water for several days and show growth of green leaves	ad libitum
Or lima beans, germinated on standing, on wet blotting-paper in a warm place for several days	ad libitum

In order to see the effect of milk as antiscorbutic feed daily over 100 gs. (mls.) of fresh milk to the control animal. The recovery is slow. After some time feed fresh carrots, onions, and germinated peas or beans; the recovery is more rapid.

D. *Production of Rickets.*—In this Exercise only healthy, young rats may be used. Secure a wooden box that is used in the shipment of fruit. The box has a board dividing it into two compartments. Remove this board and saw or bore a hole through it large enough to permit white rats to pass. Replace the board. Make a front, that can be opened, from expanded metal lathing or 1 cm. wire-mesh screening. Fill a pint milk bottle with fresh water and stopper it with a cork bearing a single glass tube that protrudes about 1 dcm., and inwardly about 0.5 dcm. With the bottle inverted, insert the tube into a small hole in the side of the cage and secure the bottle in a slightly inclined position by means of wires. The tube should be at such a height that the rat may remove drops of water from the tip of the tube by stretching its body upward, but does not brush the water from the tube in its ordinary movements about the cage. Lay a floor of sterilized (autoclaved) "excelsior" or other packing. Place the cage in a well-ventilated, but quiet and partially darkened room. Introduce a pair of breeding white rats[1] into the cage. Young will ordinarily be born (litters of from 6 to 8) within twenty-two days. The male should be left in the cage during the breeding season and until after the birth of the young. It will not disturb the young rats unless dietary or environmental conditions are abnormal. The mother must be fed a full diet of milk, cocoa, oats,

[1] White rats are for sale in various laboratories. The Wistar Institute, 36th Street and Woodland Avenue, Philadelphia, Pa., M. J. Greenman, M. D., Director, will supply stock at about $3.50 per pair. One should not use a supply of white rats of unknown origin on account of prevalence of disease, vermin, and "wildness." White rats that have mingled with the wild Norway rat may have become infected with a Trypanosome, which, introduced into the human body, leads to a sleeping sickness of the general nature of the disease of the Congo. In the case of rat-bite fever the same treatment administered for trypanosomiasis is effective.

cracked whole wheat, fresh vegetables, like chard, lettuce, carrots, cabbage, and apples. Cocoa should be added as a variation from a monotonous diet. Germinated beans of cereals should always form a part of the general diet of the male and female. The food should be administered three times daily during the lactating period and the excess food removed. After thirty days, when the young have reached a weight of about 50 gs., the experiment leading to rickets may be begun. Isolate the young animals in groups of two to four and feed exclusively the following diet (Steenbock, 2965):[1]

	Parts.
Yellow corn	76
Wheat gluten	20
Calcium carbonate	3
Sodium chlorid	1

This diet will ordinarily produce rickets in the white rat within a month. Within about this time rickets will become active, manifesting itself by lameness. Kill one rat and, if possible, photograph it with the Roentgen ray at the knee-joint upon a dental film (pages 644, 645). Section as for the conditions in scurvy (page 879, note 2). To the remaining rats feed an antirachitic diet as follows. Divide the animals into two groups:

Group A receives standardized[2] cod-liver oil in liberal amounts in addition to the rachitic diet just given.

Group B receives irradiated cholesterol[3] or lanolin containing cholesterol esters.

Improvement should be noticed after a few days. The progress of calcification should be followed if possible by the fluoroscope or by roentgenograms. After several weeks kill one rat and section the head of the femur (page 648), as previously directed (page 879, note 2).

Exercise 32.—At this point each student should record his diet, so that it may be referred to in evaluating his caloric intake. Record the complete diet for breakfast, dinner, and supper (or lunch and dinner), as well as food ingested between meals. Record all intake

[1] Personally communicated by Dr. Steenbock to the author; the favor shown in this instance is greatly appreciated.

[2] That is, commercial cod-liver oil, the antirachitic value of which is indicated on the label.

[3] Cholesterol that has been exposed to the rays of an ultraviolet quartz lamp at a distance of 10 cms. for over two hours. The Cooper-Hewitt lamp may be used. See page 649.

of water (a glass holds from 150 to 200 mls. according to the kind of glass), beverages, etc. Continue for one week.

EXERCISE 33.—Qualitative determinations on pathological urines: Secure from the instructor pathological urine. Make tests for the following substances:

1. Albumin (pages 763, 1 and 764, 3).
2. Sugar (page 161).
3. Aceton (page 781).
4. Di-acetic acid (page 781).

EXERCISE 34.—Quantitative determination of protid in the urine: Use the Quick method, page 765. Also use the Esbach method, page 231. Check your results by the method given on page 766, weighing the precipitated protid.

EXERCISE 35.—Determine the sugar content as glucose of the specimen of urine used in a preceding Exercise (Exercise 33) by the two following methods:

1. Method of Folin (page 773).
2. Method of Benedict (page 773).

EXERCISE 36.—Make osazone tests upon the unknown urines given you.

EXERCISE 37.—Under the direction of the instructor make polariscope analysis of the unknown urine given you in Exercise 33.

EXERCISE 38.—*Effect of Insulin.*—This Exercise should be commenced at the beginning of a laboratory period in order to give adequate time for the completion of the recovery stages. A white (albino) rabbit is preferred. Weigh the rabbit which has been starved for twelve hours preceding the experiment. Clip the hair from the outer margin of one ear and incise the marginal vein (Fig. 258). By massage aid the flow of blood and collect about 3 to 4 mls. of blood. A chemist, selected from the group of students,[1] determines the content of blood-sugar by one of the methods outlined on pages 776–778 of the text. Inject subcutaneously (this is facilitated by holding up the skin of the back) a dose of insulin calculated to give 0.04 g. blood sugar per 100 mls. of whole blood within

[1] In large classes one rabbit should be used for each ten students; from this group an operator, a chemist, and four reporters should be selected.

four hours;[1] or a femoral vein may be exposed and the injection made into it. Withdraw blood from the marginal vein[2] at the end of one hour and again at the end of two hours, and analyze for sugar. When convulsions appear inject immediately 10 mls. of warm 1 per cent. glucose solution intravenously; or one may introduce a stomach-tube with a "physiological funnel"[3] and administer a quantity of warm 5 per cent. glucose solution into the stomach. Note the recovery. A final blood-sugar determination should be made.

Fig. 258.—Intravenous injection of a rabbit, as in insulin experimentation. (From Kolmer, Infection, Immunity, and Biologic Therapy.)

Exercise 39.—*The Piqûre of Claude Bernard.*—Introduce by stomach-tube[4] into a fully grown female rabbit 1 g. of urethane per kilo of body weight (Appendix). After thirty minutes introduce into the occiput a thin hat-pin or tempered wire, directed downward and tipped slightly forward. Note when the instrument has reached

[1] This is calculated according to the weight of the rabbit and the strength of the insulin used. It is possible that a clinical unit will be used in which one unit is contained in either 10, 20 or 40 mls., being "U-10," etc. The original Toronto unit of insulin is three times the strength of the clinical unit and the Toronto unit is defined as the amount of insulin which will produce hypoglycemia to 0.04 g. glucose per cent. and hypoglycemic convulsions in four hours. The statement made above applies to the "Iletin" of Eli Lilly & Co., Indianapolis, Ind. See Appendix.

[2] If the blood has clotted in one vein, another may be used, or the femoral or the jugular may be used.

[3] By this is meant a small, long-stemmed funnel of about 10 mls. capacity. The term is also used for the type shown in Fig. 267.

[4] A male catheter with physiological funnel and mouth-piece serves for this purpose.

the floor of the cranium. The distance should be marked off by measurement along the side of the animal's head before the experiment has begun. Move the point backward and forward and then withdraw the probe. There should be no loss of blood. Lay the animal on its back on a board and catheterize the bladder. Test the urine from time to time for reducing substance. Glucose should appear within half an hour.

EXERCISE 40.—Secure from the store-room a sample of diabetic human urine. Determine the following quantities on the same specimen:

Total nitrogen (page 697). Folin's method.

Sugar (page 776).

From this data obtain the D/N ratio.[1] Correlate the value of the ratio with the condition of the patient as obtained from the hospital record room.

EXERCISE 41.—Repeat Exercises 25, 26, 27, and 28, and analyze the blood of the subjects volunteering for this purpose for the following quantities:

1. Non-protid nitrogen.
2. Urea nitrogen.

EXERCISE 42.—By means of the Van Slyke method (page 76), under the direction of an instructor, determine the carbon dioxid combining power of blood taken from a volunteer. Is the finding normal?

EXERCISE 43.—*Gastric Analysis.*—A number of volunteers[2] abstain from breakfast and follow the routine given on page 440. The following determinations are to be made:

1. Total acidity (page 444).
2. Free acidity (page 445).
3. Organic (lactic) acid (page 450).
4. Peptic acitvity (pages 450 and 451).

[1] Page 506.

[2] Practically all students in the classes of Jefferson Medical College submit themselves to this Exercise during the term. The student must remember that this procedure is routinely performed in hospitals and involves thousands of determinations a week throughout the world. Each medical student should have experienced as many of the routine procedures in medicine as possible, since otherwise it is impossible for him to treat his patients in a sympathetic and efficient manner.

Appropriate curves will be drawn and the results discussed. (See page 448.)

EXERCISE 44.—Collect specimens of urine from the subjects in Exercise 43. Correlate the findings with those of gastric acidity. Is there any evidence of "alkaline tide"?

EXERCISE 45.—*Duodenal Drainage.*—A subject (page 462), either student or instructor, will take the duodenal tube and bile-drainage will be made. The bile should show characteristic A, B, and C biles.[1] The class will make chemical tests on the fresh human bile thus obtained:

1. Note physical characteristics:
   (*a*) Color.
   (*b*) Odor.
   (*c*) Specific gravity.
   (*d*) Foam test (page 116, Ex. 7).
   (*e*) Hay's test (page 116, Ex. 7).
2. Chemical studies:
   (*a*) Gmelin's test (page 478).
   (*b*) Pettenkofer's test (page 474).

EXERCISE 46.—Examination of specimens of body fluids for the presence of blood (pages 118, Ex. 9; 385):

EXERCISE 47.—*Glucose Tolerance Test.*—Volunteers present themselves without breakfast for this test. Each takes 100 gs.[2] of pure glucose dissolved in enough water to permit ready administration. The urine is voided immediately, at half-hour intervals for a period of two hours, and each specimen is tested for sugar. If sugar appear, quantitative determination should be made.

EXERCISE 48.—Volunteers present themselves for the following test without breakfast. Five mls. of blood are withdrawn and the time noted. Immediately 100 gs. of pure glucose are administered as in the preceding Exercise. Blood-sugar is studied on the initial blood sample and on one taken one, two, and three hours after ingestion. Plot a curve showing the blood-sugar level at the different times of the analysis.

[1] See Lyon, cited on page 487; also see Fig. 155, page 463.

[2] It is better to use the amount of glucose suitable for the weight of the subject, that is, 1.75 g. per kilo body weight.

EXERCISE 49.—*Basal Metabolism.*—The subject volunteering for this Exercise presents himself in the morning after a light dinner of the previous evening and without breakfast. He has retired early (before 10 P. M.), and comes to the laboratory, where he reclines on a cot at absolute rest for half an hour. Then the apparatus is rolled into place, after having been carefully checked for leaks or other errors, the mouthpiece adjusted, and the run is made. Eight or ten minutes suffice. Discontinue the run, make the calculations, and express the result as percentage above or below the normal, allowing a variation of ± 15 per cent., before considering the rate indicative of a pathological condition.

EXERCISE 50.—The subject of the preceding experiment is given a breakfast and the class estimates the caloric, vitamin, keto-antiketogenic value, and whatever other data can be determined within the time limit of the Exercise.

EXERCISE 51.—*Food Surveys.*—In order to instruct the prospective medical student in practical analysis of foods and diets, the following Exercise should be performed: The class is divided into groups of four students each, each group selecting a topic from the following list, and presenting the results as a paper before the class before the end of the term:

1. Estimation of Calories on own diet.
2. Estimation of Calories on diet of selected case of large individual at hard, manual labor, such as a gardener, railroad trackman, woodsman, foundryman, or coal-heaver.
3. Estimation of Calories on diet of sedentary worker, such as librarian, clerk, cashier, or draftsman.
4. Study of vitamin content in food displayed at a given market or grocery.
5. Using tables from books on diets (pages 594 and 634), estimate the salt (NaCl) content of a restaurant meal served in a selected restaurant.
6. Estimation of keto-antiketogenic value of own diet.
7. Comparison of two selected restaurants regarding cost of food as served per caloric and other nutritional values.
8. Composition of a diet for a subject suffering with gout.
9. Selection of foods from a given menu suitable for a diabetic.
10. Selection of foods from a given menu suitable for an obese person.

Other topics of similar kind may be suggested by the instructor.

APPENDIX

REAGENTS REQUIRED IN THE EXERCISES OF THE PRECEDING PAGES

Acetic Acid.—Acetate solution for amino-acid analysis, see page 912.

Acetic Acid, Glacial.—99 per cent. acetic acid. Specific gravity 1.48. Common impurity, H.COOH.

Acetic Acid, Normal Solution.—Dilute 54 mls. glacial acetic acid up to 1000 mls. with distilled water. Percentage dissociation in decinormal solution, at 25° C., 0.013 per cent.

Acetic Acid, Strong.—36 per cent. acetic acid. A commercial product.

Acetic Anhydrid.—For cholesterol determinations a freshly distilled product is desirable. Method of procedure: Weigh approximately 120 gs. fused sodium acetate and place in a liter distilling flask connected with a long Liebig condenser. Add, by means of a dropping funnel inserted through the stopper of the flask, 90 gs. acetyl chlorid, technical. Make the additions of the chlorid slowly while the flask rests in cold water, or in an ice-mixture. Mix by rotating the flask. Then place the flask on an asbestos gauze over a Bunsen burner, and when the thermometer, passed through a second hole in the stopper, reaches 130° C., collect the distillate. B. p. of acetic anhydrid, 139° C. Discard the distillate above 140° C. The chief impurities are chlorids and compounds like thioacetic acid.

Aceto-acetic Acid.—This substance may be obtained from ethyl aceto-acetate, $CH_3COCH_2.COO.C_2H_5$, by hydrolysis. The Gauther method for the synthesis of the ester, ethyl-aceto-acetate (see Meyer and Jacobson, Organischen Chemie, I, p. 961, 1893), is as follows: From a block of sodium metal shave about 30 gs. into a liter flask connected with a Hopkins reflux condenser (Fig. 112, page 293). Add 300 gs. of "absolute" acetic ethyl ester ("ethyl acetate" or "acetic ether"); a violent reaction usually occurs. If an evident reaction does not take place, very gently warm the flask. When the action has slowed, place a water-bath under the flask and continue heating as long as particles of metal sodium appear when the flask is agitated. Cool. Add dilute hydrochloric acid to distinct acidity (litmus paper). Now add one volume of saturated NaCl solution. Transfer the solution to a separatory funnel and separate the oily layer (sodium ethyl aceto-acetate) from the aqueous layer. Leave the oily layer in a desiccating dish over dry $CaCl_2$ for a day or longer. Transfer to a liter distillation flask connected with a Liebig condenser and collect the distillate that appears when the thermometer reaches 160° C. Ethyl-aceto-acetate comes over between 175° to 185° C. The yield is about 120 gs. Aceto-acetic acid from ethyl aceto-acetate: While the solution in alcohol of the acetate may be used for the tests for diacetic acid, one may convert the ethyl-ester into the acid by hydrolyzing it, but the acid decomposes in a heated acid solution: Make the potassium salt of ethyl-aceto-acetate by treating the preparation synthesized above with a 3 per cent. KOH solution and leaving the preparation in an ice-box over night. Then acidulate with dilute sulphuric acid and shake the flask with ethyl ether. Separate the ether layer from the second layer and transfer the ether to a large evaporating dish; leave on a water-bath or electric hot-plate until the ether has evaporated. Add barium carbonate solution; the sulphuric acid is removed as barium sulphate. The ester remains as a syrupy mass which absorbs water readily.

Aceto-acetic Acid Solutions for Arnold's Method.—(1) To 25 mls. of water add 2 mls. concentrated HCl and then add 1 g. *p*-amino-acetophenon. Make up to 100 mls. with water. (2) One g. sodium nitrite made up to 100 mls. H_2O. For use, mix 2 volumes of (1) with 1 of (2), add 1 volume of urine, 2 drops of concentrated NH_4OH, and to 2 mls. of the mixture add 15 mls. of concentrated HCl. A purple color indicates a positive reaction.

Aceton Solution of Scott-Wilson.—Silver mercuric cyanid solution. Dissolve 9 gs. of NaOH purified by alcohol and 0.5 g. mercuric cyanide in 60 mls. of distilled water. Add 20 mls. of a silver nitrate solution containing 0.7268 g. silver nitrate per 100

Fig. 259.—"Acid mouth" reagent bottle. In use, grasp the stopper between the index- and second finger and hold the stopper while using the contents of the bottle; do not lay the stopper upon the table.

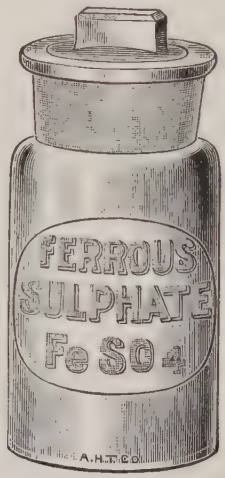


Fig. 260.—"Salt-mouth" bottle, suitable for solid chemicals, like NaCl, etc.

mls. of solution stirring slowly. If necessary, filter through a layer of asbestos in a Gooch crucible. The silver nitrate solution must be added slowly and with uninterrupted and vigorous stirring. The final solution must be clear; if turbid, let settle for several days and use the supernatant liquid decanted from the residue.

Aceton Solution for the Van Slyke Procedure.—(1) Cupric sulphate solution: 200 gs. of crystals dissolved in water and made up to 1000 mls. (2) Mercuric sulphate solution: 73 gs. of pure red mercuric oxid, dissolved in 1000 mls. of 4 normal H_2SO_4 solution. (3) Sulphuric acid: To 500 mls. of distilled water in a large flask add, cautiously, 600 mls. of concentrated sulphuric acid. Cool under the tap and make up to 1000 mls. Titrate 2 mls. against a normal NaOH solution and adjust to make a solution 17 normal. (4) Calcium hydroxid solution: Mix 100 gs. of light calcium hydroxid with 1000 mls. of distilled water. (5) Potassium dichromate solution: Dissolve 50 gs. of the crystals in water and make up to 1000 mls.

Aceton Solution for Use as Standard for Quantitative Determination.—See page 914.

Acree-Rosenheim Reagent.—1:6000 solution commercial formalin.

Alcohol, Detection of Methanol.—See page 919.

Alcoholic Sodium Hydroxid Solution.—Weigh 20 gs. of NaOH (or KOH) on a laboratory balance and dissolve in enough "absolute" ethanol to make 1000 mls.

Aldehydes, Schiff's Reaction for.—See page 923.

Alkaline Molybdate Solution.—See page 891.

Alkaline Picrate.—See page 891.

Almén's Reagent.—Dissolve 4 gs. of tannic acid in 8 mls. of 30 per cent. acetic acid and add 190 mls. of 50 per cent. ethanol.

Alpha-naphthol Solution.—Dissolve 1 g. of alpha-naphthol in about 50 mls. of ethanol and dilute with this strength alcohol to 100 mls.

Amino-nitrogen Tables.—See page 928.

Ammoniacal Silver Nitrate Solution.—See page 924.

Ammonium Carbonate Normal Solution.—48 gs. $(NH_4)_2CO_3$ dissolved in 200 mls. distilled water and made up to 1000 mls.

Ammonium Chlorid Normal Solution.—214 gs. NH_4Cl dissolved in distilled water and made up to 1000 mls.

Ammonium Hydroxid, Concentrated.—"Ammonia water." Twenty-eight per cent. NH_4OH gas dissolved in water at 20° C. Clear and colorless, specific gravity, 0.90. Common impurity, CO_2, Ca, pyridin (unless specially purified).

Ammonium Hydroxid, Dilute.—Ten per cent. solution made from the 28 per cent. by dilution. Specific gravity, 0.96.

Ammonium Hydroxid Normal Solution.—52.5 mls. of concentrated NH_4OH made up to 1000 mls. with H_2O. Percentage dissociation in decinormal solution at 25° C., 0.013 per cent.

Ammonium Molybdate Solution for Phosphates.—Dissolve 124 gs. $(NH_4)_6Mo_7O_{24}.4H_2O$ in lukewarm water and add distilled water to make 1000 mls. of solution.

Ammonium Molybdate Stock Solution.—Pour 100 mls. of 20 per cent. or stronger ammonium hydroxid solution into a mortar and add 75 gs. ammonium molybdate crystals; the preparation should be made in a hood. Grind with a pestil until the crystals are dissolved. Add 200 mls. more of the ammonium hydroxid. Pour into a 3000-ml. volumetric cylinder 900 mls. of concentrated nitric acid. Dilute with 400 mls. of distilled water. To this mixture add, in small quantities at a time, the ammonium molybdate solution first prepared. Keep the solutions cool during the additions. Finally add 1600 mls. of distilled water and mix thoroughly. Stopper. Filter if necessary.

Ammonium Nitrate Solution, Normal.—Add 80 gs. NH_4NO_3 to about 200 gs. distilled water and make up to 1000 mls.

Ammonium Oxalate Solution.—For Ca determinations. Two gs. ammonium oxalate made up to 100 mls. H_2O.

Ammonium Sulphate Standard Nitrogen Solution.—See page 913.

Ammonium Sulphide Solution.—The solution as made and sold on the market contains about 8 per cent. sulphur.

Amylol Mixture for Babcock Determinations.—Mix 50 mls. amylol (amyl-alcohol) with 50 mls. concentrated HCl.

Anilin Acetate Paper.—Suspend 1 volume of anilin in 1 volume of distilled water and 1 of acetic acid (glacial). Mix. Dip filter-paper strips into the mixture, drain, dry in the air, and preserve in salt-mouth bottles (Fig. 260).

Antifoaming Mixture.—See page 916, cetyl alcohol.

Apomorphin Hydrochlorid.—Emetic: Dog, by hypodermic syringe, 1 mg. per kilogram of body weight; or 0.1 ml. of a 1 per cent. aqueous solution per kilo. Time before effect occurs: about five minutes. Minimal dose, 0.2 mg. per kg. For cat, dose may be twice as great. Man (following accidental poisoning): 10 mgs. by mouth; 5 mgs. by hypodermic administration.

Aqua Regia (Nitrohydrochloric Acid).—Concentrated HNO_3, 1 volume; HCl, 3 volumes.

Atropin Sulphate.—Used on experimental animals to save cessation of heart-beat in overanesthetization with chloroform. Dose, rabbit, by stomach-tube, 1.5 g. Hypodermically, 500 to 750 mgs. Intravenous, 70 to 75 mgs. (All per kilo of body weight.) Dog, hypodermically, per kilo: 140 to 400 mgs. Cat, per kilo hypodermically, 30 mgs. Guinea-pig, 600 mgs. per kilo. White rat, 2.5 gs. hypodermically, per kilo. Maximum non-fatal dose for man, 1 mg.

Barfoed's Reagent.—Dissolve 45 gs. of neutral, crystal cupric acetate in about 900 mls. of distilled water. Filter if the solution is not clear. To the clear solution add 2 mls. of 30 per cent. acetic acid. Dilute with distilled water to 1000 mls.

Barium Chlorid Solution, Normal.—Add 122 gs. $BaCl_2.2H_2O$ to water and make up after dissolution to 1000 mls.

Barium Hydroxid, 0.25 Normal Solution.—4.2 gs. $Ba(OH)_2.8H_2O$ dissolved in distilled water and made up to 100 mls.

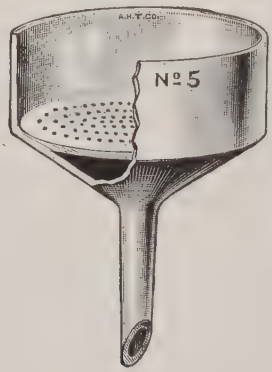


Fig. 261.—Buchner funnel. Filter-paper is laid upon the perforated disk. The stem of the funnel is inserted into the stopper of a suction flask.

Barium Hydroxid Solution, Commercial Solution.—3.3 per cent. $Ba(OH)_2.8H_2O$, aqueous solution.

Baryta Mixture.—Make the following normal barium chlorid solution: Weigh 122 gs. of $BaCl_2.2H_2O$, and make up to 1000 mls. with distilled water. To each volume of this solution add 2 volumes of the baryta water described below.

Baryta Water.—Dissolve 100 gs. of barium hydroxid ($Ba(OH)_2.8H_2O$) crystals in 1500 mls. of boiling water. Filter while hot. Permit the solution to cool. Crystals of barium hydroxid separate. Decant the supernatant liquid ("baryta water") into a bottle bearing a trap containing moist soda-lime (sodium-calcium hydrate) in order to prevent the entrance of CO_2 into the bottle.

Basic Lead Acetate.—See page 919.

Bell-Doisy Reagents for Phosphates.—See page 921 and below, (Benedict's solutions).

Benedict's Creatinin Standard Solution.—Dissolve 100 mgs. pure creatinin in distilled water in a 100-ml. volumetric flask, add 1 ml. concentrated HCl and make up to the mark with distilled water. One ml. = 1 mg. creatinin.

Benedict's Decolorizer for Creatin Determination.—Powdered lead, chemically pure.

Benedict's (with Bock) Method for Nessler's Solution.—Weigh 100 gs. of mercuric iodid HgI_2; transfer to a liter volumetric flask. Add 70 gs. of potassium iodid, KI. Cover with about 400 mls. of H_2O. Dissolve by agitating the contents of the flask. Add, with constant shaking, about 500 mls. of 20 per cent. NaOH solution and make up

to the mark with distilled water. Leave several days for a precipitate to settle, from which the clear, supernatant fluid may be decanted into another vessel.

Benedict's Method for Purifying Acid for Blood and Urine Chemistry.—Weigh 400 gs. of commercial picric acid with 10 or 20 per cent. water for the purpose of prevention of explosion. Transfer to a 2-liter Pyrex flask. Add 1 liter of chemically pure benzene, C_6H_6. Place the flask carefully on an electric hot plate, protected by a soft asbestos mat. Boil vigorously and at intervals agitate the contents. Fold a large filter-paper and place it in a funnel. Moisten the interior of the paper with benzene and carefully pour the hot picric-benzene solution from the flask through the paper into a large beaker. Place the beaker on the hotplate, cover with a watch-glass, and heat until the picric acid has been entirely dissolved. Turn off the current and let the beaker remain either on the plate or in a locker overnight. Drain off the supernatant benzene from the large, hard yellow crystals and wash twice with portions of benzene, 75-ml. portions. Transfer the crystals to a shallow dish and place on the hot plate to cause the evaporation of the benzene. The temperature must not be over

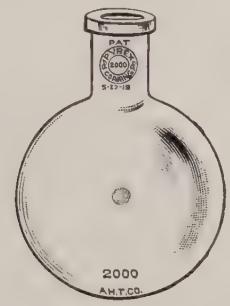


Fig. 262.—"Balloon type" flask for use in continuous boiling.

80° C. Keep the crystals thus purified and dried in a salt-mouth bottle. Remember that picric acid is highly inflammable and explosive when contained in an enclosed vessel; even a mechanical jar is sufficient sometimes to cause explosion.

Benedict's Molybdic Acid Reagent for Blood Phosphorus (compare the sulphite reagent mentioned below).—Dissolve 20 gs. of pure, ammonia-free molybdic acid, MoO_3, in 25 mls. of 20 per cent. NaOH. Warm to about 50° C. Dilute to 200 mls. with distilled water. Mix and filter. Transfer to a liter flask and add, while agitating the contents of the flask, under cold water, from the tap, 200 mls. of concentrated sulphuric acid. The color must not be brownish, for this indicates that the solution has become overheated during the addition of the acid.

Benedict's Picric-picrate Solution.—To 125 mls. of normal sodium hydroxid solution, in a liter volumetric flask, add about 700 mls. of distilled water at 80° C. Add 36 gs. of dry or 40 gs. moistened picric acid and dissolve completely by agitating the contents of the flask. Cool to room temperature and make up to the mark (1000 mls.). Filter.

Benedict's Reagent for Blood Phosphorus.—Dissolve 30 gs. of sodium bisulphite, $NaHSO_3$, in about 100 mls. of distilled water in a 200-ml. volumetric flask. Add 1 g. hydrochinon and mix. Dilute to the mark with H_2O.

Benedict's Reagents for the Direct Method for Uric Acid in Blood and Urine.—(1) Place 100 gs. of sodium tungstate[1] in a liter Pyrex flask. Add about 600 mls. of distilled water. Dissolve. Add 50 gs. pure arsenic pentoxid, As_2O_5. Dissolve. Add 25

[1] Specially prepared for blood and urine analysis. Several of the larger house furnishing chemicals will supply this material.

mls. of 85 per cent. phosphoric acid and 20 mls. of concentrated HCl. Boil for twenty minutes, cool, transfer to a liter volumetric flask, and make to the mark with distilled water.

(2) Weigh 5 gs. of NaCN and add enough water to make 100 mls. of solution.

(3) Specially diluted Benedict-Hitchcock uric acid standard solution (below): Pipette 50 mls. of the Benedict-Hitchcock standard solution into a 500-ml. volumetric flask. Dilute to about 400 mls. with distilled water. Add 25 mls. of 1:10 HCl solution, dilute to the mark, and mix. Ten mls. of this standard solution contain 0.2 mg. of uric acid.

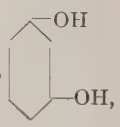
Benedict's Solution for Sugar, Quantitative.—Dissolve 173 gs. of trisodium citrate ($Na_3C_6H_5O_7.11H_2$) in about 600 mls. of distilled water at about 60° C. Add 90 gs. of dry sodium carbonate, Na_2CO_3. Filter through a fluted filter. Make up to 850 mls. with distilled water. Weigh exactly 17.3 gs. of highest purity crystallized cupric sulphate ($CuSO_4.5H_2O$) and dissolve in about 100 mls. of distilled water. After mixing, dilute to about 150 mls. To the citrate-carbonate solution add, with constant stirring and in small amounts, the cupric sulphate solution. The volume should be close to 1000 mls.

Benedict's Solution for Sugar, Quantitative.—To about 600 mls. distilled water at 60° C. add 200 gs. of trisodium citrate. Dissolve by stirring. Add 200 gs. crystallized (or 75 gs. anhydrous) sodium carbonate. Finally, weigh 125 gs of potassium thiocyanate (KCNS) rapidly from a stock bottle and dissolve in the above solution of citrate-carbonate. Dilute with distilled water to about 800 mls. Let stand until cool and then filter. Weigh carefully 18 gs. of $CuSO_4.5H_2O$ crystals and dissolve in about 100 mls. of distilled water. Pour this solution slowly and with stirring into the citrate-carbonate-thiosulphate solution first made. Add 5 mls. of 5 per cent. solution of potassium ferrocyanide ($K_4Fe(CN)_6.3H_2O$). Dilute with distilled water to 1000 mls., filter, and keep in cork-stoppered bottles.

Benedict's Standard Glucose Solution.—(1) For urine: Dissolve 167 mgs. pure glucose in 400 mls. distilled water. Add 5 mls. of toluene, mix well, and dilute to 500 mls. with distilled water.

(2) For blood: Dissolve 0.1 g. pure glucose in about 400 mls. of 2 per cent. picric acid in a 500-ml. volumetric flask. Mix and dilute to the mark with the saturated picric acid. Three mls. of this solution = 0.6 mg. glucose.

Benedict's Standard Phenol Reagent.—Weigh 11.62 mgs. of pure resorcinol,



and dilute to 100 mls. with 0.1 normal HCl. 5 mls. = 0.5 mg. phenol.

Benedict's Standard Phosphorus Solution.—Dissolve 0.4394 g. of potassium dihydrogen phosphate in a liter volumetric flask containing about 800 mls. of distilled water and make to the mark with chloroform water (10 mls. $CHCl_3$ shaken up with 200 mls. H_2O). For use, pipette 5 mls. of this stock solution into a 100 ml. flask and make up to the mark with chloroform water. Each 5 mls. of this diluted solution contains 0.025 mg. of phosphorus.

Benedict's Total Sulphur Reagent.—Into a 100-ml. volumetric flask place 20 gs. of cupric nitrate crystals. Add 50 mls. of distilled water, lukewarm (50° C.). Then add 5 gs. of potassium chlorate, $KClO_3$, and make to the mark with distilled water.

Benedict-Hitchcock Standard Uric Acid Solution.—Dissolve 9 gs. of pure Na_2HPO_4 and 1 g. NaH_2PO_4 in 250 mls. of distilled water at 60° C. Filter into a 500-ml. volumetric flask and make up to the mark with cold water. Pour this tepid solution over exactly 200 mgs. of uric acid suspended in a few mls. of distilled water in a liter volumetric flask. Agitate the contents of the flask until all of the uric acid has become

dissolved. Cool, add exactly 1.4 ml. glacial acetic acid and 5 mls. of chloroform. Then dilute to the mark. Five mls. contain 1 mg. uric acid. Keep in a cool place.

Benedict-Nash Reagent for the Ammonia Content of the Blood.—Into a Pyrex beaker of 100 mls. capacity weigh 8 gs. of potassium carbonate, K_2CO_3, and add 50 mls. of distilled water. After solution of the carbonate add 12 gs. of potassium oxalate crystals. Boil to about 30 mls. and then dilute to about 50 mls. with ammonia-free distilled water.[1] Repeat once and then finally dilute to 80 mls. Keep well stoppered.

Benedict-Osterberg Boneblack for the Determination of Normal Urinary Sugar.—Method of purification: Treat 250 gs. of commercial boneblack with 1500 mls. of 1:4 HCl solution. Boil for half an hour. Filter the boneblack from the acid solution by

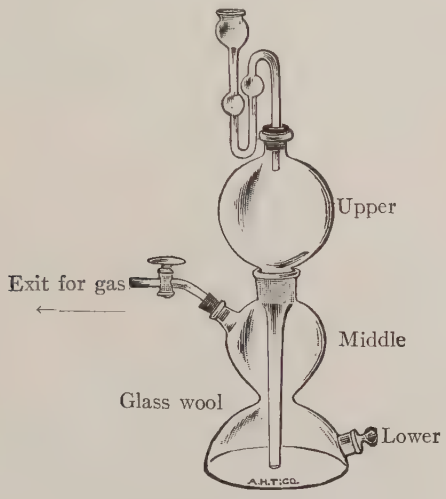


Fig. 263.—Kipp gas generator.

means of a Buchner funnel and aspirating pump. Wash with hot water until the washings are neutral to litmus paper. Dry and powder. Test: Shake 15 mls. of a 0.5 per cent. glucose solution with 1 g. of the boneblack; filter and make a test for glucose on the filtrate. The test must be negative.

Benzidin Solution for Sulphates.—Grind 4 gs. of best quality benzidin with about 10 mls. of distilled water in a mortar. Wash into a 2000-ml. flask by means of about 500 mls. of distilled water. Add 5 mls. of concentrated hydrochloric acid and make up to the mark.

Hydrochloric acid 25 per cent. solution: Dilute 1 volume of concentrated hydrochloric acid with 3 volumes of distilled water.

Saturated benzidin sulphate solution: Make a solution of benzidin sulphate by adding 5 gs. of sodium sulphate to 200 mls. of the benzidin hydrochlorid solution described above. Filter through a hardened filter in a Buchner funnel with suction, saving the residue on the paper. Wash once with cold water. Add the residue to 500 mls. of distilled water at 60° C. Permit to stand over night in a cool place. Filter through ordinary paper in a funnel. The solution must be clear.

Bial's Reagent.—Add 1.5 gs. of orcinol to 500 mls. of concentrated hydrochloric acid. Mix and add 6 drops of 5 per cent. solution ferric chlorid.

[1] Distil in resistance glass (Pyrex, Jena, quartz) 250 mls. of freshly distilled water with 10 mls of concentrated H_2SO_4, saving only the middle portion.

Bichromate.—See page 922.

Bigwood-Ladd Nitroprussid Reagent for Aceton.—To 10 mls. of acetic acid, glacial, add 10 mls. of a 10 per cent. solution of sodium nitroprussid (sodium nitroferrocyanid). Store for use (not over 2 weeks) in an amber bottle, well stoppered.

Boneblack Preparation for Sugars in Normal Urine.—See page 893.

Bromin Poisoning Treatment.—Apply anilin; in case of overbreathing Br fumes, inhale anilin fumes for not over thirty seconds (half a minute) at a time.

Bromin Water.—Place a large flask containing 100 mls. of distilled, cold water in a hood, or under an exhaust. Add a few drops of liquid bromin from a stock bottle and replace the stopper at once. Shake the flask vigorously. Add more bromin and repeat. Caution: Bromin affects the respiratory mucous membrane. In case of excess breathing of the bromin fumes, snuff into the nostrils a small amount of anilin vapors. In case a bromin burn occurs, remove the excess bromin by means of a piece of cotton and apply anilin. Anilin bromid is formed which is less irritating than bromin.

Fig. 264.—Allihn type condenser. This form of condenser is especially desirable in biochemical work.

Bruecke's Reagent.—Dissolve 50 gs. of potassium iodid in 500 mls. of distilled water, saturating with mercuric iodid (120 gs.). Make up to 1000 mls.

Buffer Solutions: Sørensen Solutions.—(1) Decinormal hydrochloric acid solution: Pour about 200 mls. of best quality commercial hydrochloric acid, concentrated specific gravity 1.18, into a distillation flask connected with a Liebig condenser. Distil until one-third has passed over. The acid solution left contains 20.2 per cent. of HCl gas. Dilute 165 mls. up to 1 liter; this will give almost a normal solution. Determine the normality by titrating against an exact standard solution of base.

(2) Decinormal sodium hydroxid solution: Obtain from the store-room 50 grams of stick sodium hydroxid from sodium. Place it immediately in a weighing bottle and weigh out, rapidly, on a torsion balance, 42 grams. Dissolve the sticks in

distilled water and make up to a liter with distilled water. Cool to room temperature. Determine the normality by titrating against standard sulphuric acid with methyl orange as indicator. Keep the solution in a vessel closed with a trap containing a saturated solution of barium hydroxid. From this stock make up a decinormal solution by dilution.

(3) Glycin-NaCl solution: Test a small amount of the glycin[1] for chlorion and sulphion; if either is present in detectable amounts, discard the preparation. By means of the Kjeldahl total nitrogen method determine the nitrogen in 2 grams of a sample; the theoretical amount of nitrogen is 18.67 gs. per 100 grams of glycin. From a pure specimen, weigh 7.505 gs. Weigh, from a pure sodium chlorid supply, 5.85 gs. NaCl. Dissolve the glycin and the salt in about 200 mls. distilled water and make up to 1 liter solution. This makes a decimolar solution of glycin.

(4) One-fifteenth molecular solution of acid phosphate: Select a specimen of mono-potassium-di-hydrogen phosphate ("primary potassium phosphate"), KH_2PO_4, that will exhibit a marked change in color when either acid or alkali in decinormal

Fig. 265.—A 50-ml. centrifuge tube, graduated. For mounting in use see Fig. 256.

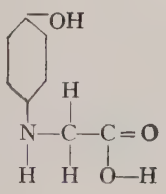
solution is added to a weak solution of the salt. The salt should be water-free (loss of weight on drying at 100° C. for twenty-four hours not over 1 part in 1000). Weigh 9.078 gs. and dilute to 1000 mls. with distilled water.

(5) Fifteenth molecular solution of alkaline phosphate: As obtained on the market, Na_2HPO_4 bears 12 molecules of water of crystallization. The Sørensen standard buffer uses the salt $Na_2HPO_4.2H_2O$. This may be purchased by special order from supply houses, or it may be obtained by exposing some of the salt with $12H_2O$ to a dust-free atmosphere for about a fortnight during which period $10H_2O$ are lost, leaving $Na_2HPO_4.2H_2O$. Tests for chlorion and sulphion must be negative. Weigh 11.876 gs. $Na_2HPO_4.2H_2O$ and dissolve in water; make up to 1 liter.

(6) Decinormal solution of sodium citrate: This solution is made by mixing citric acid with sodium hydroxid: Crystals of the acid,

$$\begin{array}{l} H_2C\text{—}COOH \\ | \\ HOC\text{—}COOH \\ | \\ H_2C\text{—}COOH.H_2O, \end{array}$$

[1] Note that this glycin (amino-acetic acid) is not the same as the glycin sold on the photographic market as "glycin," which is:



dissolved in water must show no qualitative test for chlorion or for sulphion. When dried in an oven at 70° C., for twenty-four hours, the color of the crystals must not change. Weigh 21.008 gs. and dissolve in exactly 200 mls. normal solution of sodium hydroxid prepared as directed under (2) above. Dilute to 1000 mls. with distilled water.

(7) Boric solution: A pure brand of boric acid, H_3BO_3, should be selected. Throw about 5 gs. of the substance into distilled water; there should be no residue. Chlorion and sulphion tests must be negative. Treated with a few drops of methyl orange solution, the color must be orange. Weigh 12.404 gs. of the acid and dissolve in exactly 100 mls. normal sodium hydroxid solution; then make up to 1000 mls. with distilled water.

Caffein for Stimulating Overanesthetized Experimental Animal.—Dog, hypodermically, per kilo, 75 mgs. By stomach-tube, 100 mgs. Cat, hypodermically, 100 mgs. per kilo. Rabbit, intravenous, 100 mgs. per kilo; hypodermically, 200 mgs. per kilo; by stomach-tube, 250 mgs. per kilo. Fatal dose for man, 500 mgs.

Casein Solution.—Suspend, by stirring, 1 g. Harris' special casein (page 110) in about 20 mls. distilled water in a beaker. Add exactly 10 mls. 0.1 normal NaOH solution.

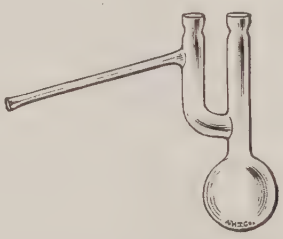


Fig. 266.—Claisen flask used in distillations. The extra tube at the side permits the introduction of a thermometer for accurately recording the temperature of the distillate. "Bumping" is avoided by inserting a small-bore tube into the straight tube, passing into the flask, nearly touching the bottom. Pieces of pumice or beads may be placed in the side tube in order to prevent droplets from actively boiling liquids in the flask from entering the distillate.

Stir. Leave until a homogeneous solution is obtained (about an hour) and transfer to a 100-ml. volumetric flask; made up to 100 mls. with water.

Chlorids in the Urine, Seelman Reagents.—See page 924.

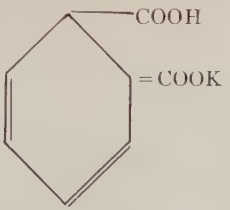
Chlorin Water.—In a Florence flask provided with thistle-tube and exit-tube pour 50 per cent. H_2SO_4 upon NaCl mixed, equal parts by weight, with manganese dioxid. Apply heat to the flask and pass the gas through water. Saturation: 0.73 g. to 100 mls. H_2O at 20° C.

Chloroform as Anesthetic.—Used only where ether cannot be used. Average amount of $CHCl_3$ necessary for anesthesia, 0.5 ml. of 0.1 per cent. solution of $CHCl_3$ per kilo of body weight in veins. Administration may be made in this amount by vein, but it is customary to introduce the chloroform by way of the respiratory system, in which case the criterion of narcosis is the loss of the corneal reflex (page 191). Frogs, 200 mgs. (1 ml. of 20 per cent. $CHCl_3$ oil solution) into the dorsal lymph sac.

Clark-Lubs Indicators.—See page 917.

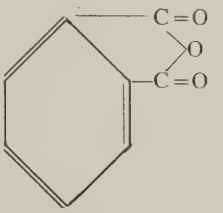
Clark-Lubs Solutions.—(I) Fifth molecular potassium chlorid solution. Recrystallize the best commercial preparation obtainable three or four times; then dry in an oven at 120° C. for about thirty-six hours. Weigh 14.912 gs. of the KCl and dissolve in water; make up to 1000 mls.

(II) Fifth molecular acid potassium phthalate: This is obtainable on the market, or one may use the method of Dodge.[1] Its structural formula is:



Acid potassium phthalate.

Clark[2] gives the following method for acid potassium phthalate from sodium hydroxid and ortho-phthalic anhydrid,



o-Phthalic anhydrid.

Dissolve 60 grams of NaOH from sodium (see Buffers, 2, above) in about 400 mls. of distilled water. Cool. Add 50 gs. freshly resublimed ortho-phthalic anhydrid. Mix. To 5 mls. of the cooled solution add a drop of phenolphthalein indicator solution; adjust the reaction to a slight pink, adding either more KOH or anhydrid as necessary, taking account of the amount of anhydrid used. Now add as much phthalic anhydrid again as the solution contains. Heat in order to cause complete solution. Filter while hot, saving the residue. Set the filtrate aside to cool slowly; crystals appear. Remove the crystals by means of a Buchner funnel and hardened paper. Recrystallize twice from distilled water. Leave in an oven at from 110° to 115° C. until the salt reaches constant weight. Weigh of these crystals 40.828 gs. Make up to 1000 mls. with water. This makes a fifth molecular solution of acid potassium phthalate.

(III) Fifth molecular solution of mono-potassium di-hydrogen phosphate, KH_2PO_4: Recrystallize the commercial preparation from water. Dry in the oven at 110° to 115° C. Weigh, after constant weight has been reached, 27.232 gs. and make up to 1 liter with water. Test the solution with methyl red; the color assumed by the KH_2PO_4 solution should be distinctly red.

(IV) Fifth molecular boric acid (H_3BO_3)—fifth molecular potassium chlorid solution: Recrystallize the boric acid twice. Dry in the air, or in a warm oven below 50° C., in thin layers. For KCl refer to (I) above. Weigh 12.4048 gs. of H_3BO_3 and dissolve in water. Weigh 14.912 gs. KCl and dissolve in the borate solution. Make up to 1000 mls. with water.

(V) Fifth molecular (or normal) sodium hydroxid solution: Clark's method is given as follows: This solution should be as free as possible from carbonate. A solution of sufficient purity may be prepared from a high-grade sample of the hydroxid

[1] Dodge, F. D. (Chemist, Dodge & Olcott Co., Bayonne, N. J.), Jour. Industrial and Eng. Chem., vol. 7, p. 29, 1915.

[2] Clark, W. M., Determination of Hydrogen Ions, 1st ed., 1920, p. 70, Baltimore, Williams & Wilkins, 1920.

in the following manner: Dissolve 100 grams NaOH in 100 mls. distilled water in a Jena or Pyrex glass Erlenmeyer flask. Cover the mouth of the flask with tin foil and allow the solution to stand overnight until the carbonate has settled. Then prepare a filter as follows: Cut a "hardened" filter paper to fit a Buchner funnel. Treat it with warm, strong [1:1] NaOH solution. After a few minutes decant the sodium hydroxid and wash the paper first with "absolute" ethanol, then with dilute ethanol (50 per cent.), and finally with large quantities of distilled water. Place the paper on the Buchner funnel and apply gentle suction until the greater part of the water has evaporated; but do not dry so that the paper curls. Now pour the concentrated alkali upon the middle of the paper, spread it with a glass rod, making sure that the paper, under gentle suction, adheres well to the funnel, and draw the solution through with suction. The clear filtrate is now diluted quickly, after rough calculation, to a solution somewhat more concentrated than normal. Withdraw 10 mls. of this dilution and standardize roughly with an acid solution of known strength, or with a sample of acid potassium phthalate. From this approximate standardization calculate the dilution required to furnish an M/5 solution. Make the required dilution with the least possible exposure, and pour the solution into a *paraffined*[1] bottle to which a calibrated 50-ml. burette and soda-lime guard tubes have been attached. The solution should now be most carefully standardized. One of the simplest methods of doing this, and one which should always be used in this instance, is the method of Dodge (1915), in which use is made of the acid potassium phthalate purified as already described. Weigh out accurately on a chemical balance with standardized weights several portions of the salt of about 1.6 grams each. Dissolve in about 20 mls. distilled water and add 4 drops phenolphthalein. Pass a stream of CO_2-free air through the solution and titrate with the alkali until a faint but distinct and permanent pink is developed. It is preferable to use a factor with the solution rather than attempt adjustment to an exact M/5 solution.

(VI) Fifth molecular hydrochloric acid solution: Dilute a high-grade hydrochloric acid solution to about 20 per cent. and distil. Dilute the distillate to approximately fifth molecular and standardize with the sodium hydroxid solution just described. If convenient it is well to standardize this solution carefully by the silver chlorid method[2] and check with the standardized alkali.

GLYCIN BUFFERS (SØRENSEN)

Glycin, mls.	HCl, mls.	pH.
0.0	10.0	1.038
1.0	9.0	1.146
2.0	8.0	1.251
3.0	7.0	1.419
4.0	6.0	1.645
5.0	5.0	1.932
6.0	4.0	2.279
7.0	3.0	2.607
8.0	2.0	2.922
9.0	1.0	3.341
9.5	0.5	3.679

[1] Clark finds that thick coats of paraffin are more satisfactory than the thin coats sometimes recommended. Thoroughly clean and *dry* the bottle, warm it, and then pour in the melted paraffin. Roll gently to make an even coat and just before solidification occurs stand the bottle upright to allow excess paraffin to drain to the bottom and there form a very substantial layer.

[2] See Sutton, F., A Systematic Handbook of Volumetric Analysis, 11th ed., Philadelphia, P. Blakiston's Son & Co., 1924, p. 183.

Glycin Buffers (Sørensen)

	NaOH, mls.	ⱡH.
9.5	0.5	8.575
9.0	1.0	8.929
8.0	2.0	9.364
7.0	3.0	9.714
6.0	4.0	10.140
5.5	4.5	10.482
5.1	4.9	11.067
5.0	5.0	11.305
4.9	5.1	11.565
4.5	5.5	12.095
4.0	6.0	12.399
3.0	7.0	12.674
2.0	8.0	12.856
1.0	9.0	12.972
0.0	10.0	13.066

Citrate Buffers (Sørensen)

Citrate, mls.	HCl, mls.	ⱡH.
0.0	10.0	1.038
1.0	9.0	1.173
2.0	8.0	1.418
3.0	7.0	1.925
3.33	6.67	2.274
4.0	6.0	2.972
4.5	5.5	3.364
4.75	5.25	3.529
5.0	5.0	3.692
5.5	4.5	3.948
6.0	4.0	4.158
7.0	3.0	4.447
8.0	2.0	4.652
9.0	1.0	4.830
9.5	0.5	4.887
10.0	0.0	4.958

	NaOH, mls.	
9.5	0.5	5.023
9.0	1.0	5.109
8.0	2.0	5.314
7.0	3.0	5.568
6.0	4.0	5.969
5.5	4.5	6.331
5.25	4.75	6.678

COMPOSITION OF BUFFERS GIVING *p*H VALUES AT 20° C. AT INTERVALS OF 0.2

KH_2PO_4—NaOH Buffers (Clark)

KH_2PO_4.	NaOH.	Dilution.	*p*H.
50 mls. M/5	3.72	To 200 mls.	5.8
"	5.70	"	6.0
"	8.60	"	6.2
"	12.60	"	6.4
"	17.80	"	6.6
"	23.65	"	6.8
"	29.63	"	7.0
"	35.00	"	7.2
"	39.50	"	7.4
"	42.80	"	7.6
"	45.20	"	7.8
"	46.80	"	8.0

Boric acid—KCl—NaOH Buffers (Clark)

Boric.	NaOH.	Dilution.	*p*H.
50 mls. M/5	2.61	To 200 mls.	7.8
"	3.97	"	8.0
"	5.90	"	8.2
"	8.50	"	8.4
"	12.00	"	8.6
"	16.30	"	8.8
"	21.30	"	9.0
"	26.70	"	9.2
"	32.00	"	9.4
"	36.85	"	9.6
"	40.80	"	9.8
"	43.90	"	10.0

KCl—HCl Buffers[1] (Clark)

KCl.	HCl.	Dilution.	*p*H.
50 mls. M/5	64.5 mls.	To 200 mls.	1.2
"	41.5 "	"	1.4
"	26.3 "	"	1.6
"	16.6 "	"	1.8
"	10.6 "	"	2.0
"	6.7 "	"	2.2

[1] The *p*H values of these mixtures are given by Clark and Lubs (1916) as *preliminary* measurements.

Phthalate-HCl Buffers (Clark)

Phthalate.	HCl.	Dilution.	*p*H.
50 mls. M/5	46.70 mls.	To 200 mls.	2.2
"	39.60 "	"	2.4
"	32.95 "	"	2.6
"	26.42 "	"	2.8
"	20.32 "	"	3.0
"	14.70 "	"	3.2
"	9.90 "	"	3.4
"	5.97 "	"	3.6
"	2.63 "	"	3.8

Phthalate-NaOH Buffers (Clark)

Phthalate.	NaOH.	Dilution.	*p*H.
50 mls. M/5	0.40 mls.	To 200 mls.	4.0
"	3.70 "	"	4.2
"	7.50 "	"	4.4
"	12.15 "	"	4.6
"	17.70 "	"	4.8
"	23.85 "	"	5.0
"	29.95 "	"	5.2
"	35.45 "	"	5.4
"	39.85 "	"	5.6
"	43.00 "	"	5.8
"	45.45 "	"	6.0
"	47.00 "	"	6.2

Phosphate mixtures (Sørensen)

Na_2HPO_4	KH_2PO_4.	*p*H.
0.25 mls.	9.75 mls.	5.28
0.50 "	9.5 "	5.58
1.00 "	9.0 "	5.90
2.0 "	8.0 "	6.23
3.0 "	7.0 "	6.46
4.0 "	6.0 "	6.64
5.0 "	5.0 "	6.81
6.0 "	4.0 "	6.97
7.0 "	3.0 "	7.16
8.0 "	2.0 "	7.38
9.0 "	1.0 "	7.73
9.5 "	0.5 "	8.04

Borate mixtures (Sørensen)

Borate.	HCl.	*p*H.
5.25	4.75	7.62
5.50	4.50	7.93
5.75	4.25	8.13
6.0	4.0	8.29
6.5	3.5	8.50
7.5	2.5	8.79
8.0	2.0	8.90
8.5	1.5	9.00
9.0	1.0	9.08
9.5	0.5	9.16
10.0	None	9.24

Borate.	NaOH.	*p*H.
9.0	1.0	9.36
8.0	2.0	9.50
7.0	3.0	9.67
6.0	4.0	9.97
4.0	6.0	12.37

Kolthoff[1] Buffer Mixtures

18° C.

Succinic acid 0.05 molar.	Borax 0.05 molar.	*p*H.
9.86 mls.	0.14 mls.	3.0
9.65 "	0.35 "	3.2
9.40 "	0.60 "	3.4
9.05 "	0.95 "	3.6
9.63 "	1.37 "	3.8
8.22 "	1.78 "	4.0
7.78 "	2.22 "	4.2
7.38 "	2.62 "	4.4
7.00 "	3.00 "	4.6
6.65 "	3.35 "	4.8
6.32 "	3.68 "	5.0
6.05 "	3.95 "	5.2
5.79 "	4.21 "	5.4
5.57 "	4.43 "	5.6
5.40 "	4.60 "	5.8

[1] Kolthoff, I. M. (Utrecht, Netherlands), Jour. Biol. Chem., vol. 63, page 135, 1925

Monopotassium di-hydrogen phosphate KH_2PO_4, 0.1 molar.	Borax 0.05 molar (as above).	*p*H.
9.21 mls.	0.79 mls.	5.8
8.77 "	1.23 "	6.0
8.30 "	1.70 "	6.2
7.78 "	2.22 "	6.4
7.22 "	2.78 "	6.6
6.67 "	3.33 "	6.8

Monopotassium di-hydrogen phosphate KH_2PO_4, 0.1 molar.	Borax 0.05 molar (as above).	*p*H.
6.23 mls.	3.77 mls.	7.0
5.81 "	4.19 "	7.2
5.50 "	4.50 "	7.4
5.17 "	4.83 "	7.6
4.92 "	5.08 "	7.8
4.65 "	5.35 "	8.0
4.30 "	5.70 "	8.2
3.87 "	6.13 "	8.4
3.40 "	6.60 "	8.6
2.76 "	7.24 "	8.8
1.75 "	8.25 "	9.0
0.50 "	9.50 "	9.2

Cleaning Fluid.—Chromic acid solution. Dissolve 100 gs. of chromic "acid" (CrO_3) in distilled water and make up to 1000 mls.

Chromic-sulphuric acid solution: Dissolve 100 gs. of potassium dichromate in enough 10 per cent. sulphuric acid solution to make a liter of solution. Note: When the solution, after use, turns green, the chromic acid (CrO_3) has become

chromic oxid (Cr_2O_3) which has lost its power of causing oxidation as readily as CrO_3 and should be discarded. The cleaning solution should be red. Moreover, the solution must be used warm (40° C.) to be effective for the usual purposes of biochemical laboratory.

CO_2 Generation.—Introduce a small mass of glass-wool into the bottom of the middle compartment of a Kipp generator (Fig. 263) and, after removing the tube and cock (gas exit), introduce enough lumps of marble or $CaCO_3$ to half fill that compartment. Remove the safety-tube from the upper compartment, open the gas-exit cock, and add 50 per cent. hydrochloric acid through the top opening until the lower compartment is filled and all of the lime in the middle compartment is covered. Close gas exit; as gas is formed the acid is displaced and rises to the upper chamber. When ready to use gas, simply open gas-exit cock.

Collodion Membranes and Tubes.—Secure Anthony's Negative Cotton from the Ansco Company, Binghampton, N. Y. Weigh out 3 gs., place in a salt-mouth bottle (Fig. 260), and pour over it 75 mls. of pure, preferably redistilled, ethyl ether. Leave half an hour, stoppered, shaking the bottle at intervals. Then add 25 mls. of "absolute" ethanol and shake the contents gently until a solution fairly transparent is obtained.

In making the membrane, or tube, cover the plate (for membrane), or fill the container (test-tube, etc.) with the collodion and then pour off all that is possible. Stand the plate against a support to drain. Clamp the vessel inverted in a clamp of a burette-stand and leave for ten minutes, but no longer. Then by aid of a knife-point and a wash-bottle (Fig. 43) remove the membrane carefully, washing the tissue from its support (glass wall of the test-tube, etc.). Preserve in distilled water.

For methods outlined by Looney see Jour. Biol. Chem., vol. 50, page 1, 1922. This solution contains ethyl acetate which so modifies the membrane that they can be used after drying.

Colloidal Gold.—Secure a supply of double distilled water: Distill ordinary distilled water from a pyrex, or quartz vessel, the water having had a half-dozen crystals of potassium permanganate dissolved in it previously. Using this water after distillation, place 1000 mls. in a 2-liter pyrex or quartz flask. Add 10 mls. 1 per cent. solution of gold chlorid made from crystals. Add, then, exactly 7 mls., of a 2 per cent. solution of potassium carbonate from a freshly opened bottle. Add 0.5 ml. 1 per cent. solution of oxalic acid. Heat this mixture after complete solution to the boiling-point of water. Remove the flask from the flame and shake the contents vigorously. Then immediately add 8 drops of commercial formalin (40 per cent. solution H.CHO gas in water). Color should develop; if it does not, reheat to boiling and then violently agitate the contents again. Add, while the solution is in motion, not over 4 drops more of the formalin solution. The color should be a deep red. Store out of the light in a pyrex or other vessel incapable of giving much alkali to the solution.

Colloidal Platinum.—Fix to the ends of a set of wires from the house-current, cut down by means of a rheostat, or lamp-set, to 30–40 volts and using 5–10 amperes, D. C., platinum wires about 2 cms. long on each terminal. Insert these wires through glass tubes about a decimeter long, to serve as handles. While submerged beneath the surface of pure, "conductivity" water, in which a minute amount of chemically pure Na_2CO_3 has been dissolved, bring the tips carefully toward one another, but never touching; a small arc will form between the tips of the wires, and after a short time there will be a brownish color appear in the water. When distinctly discolored with this brown platinum "sponge" (partly coagulated platinum sol.), discontinue the procedure. For an improved process see Svedberg, T., Herstellung Kolloider Lösungen.

Colorimetric Calculation; Check on the Beer's Formula (after McCraken, et al.)

The following two tables may be employed to check colorimetric readings; the *unknown* is set at 20 mms. D means dividend, the decimal fractions being 0.0, 0.1, 0.2, etc., across the top. Thus the standard may read 13.3 mms., in which case the quotient is 0.665. For instance, for an analysis of blood-sugar by Folin's method:

The weight of glucose used as the standard is 0.4 mg., the reading for the known is 19.7; when the unknown is set on 20, find the number of milligrams of glucose in 100 cc. of blood. The general formula applicable is:

$$\frac{\text{Reading on Known}}{\text{Reading on Unknown}} \times \frac{\text{Vol. Unknown}}{\text{Vol. Known}} \times \frac{\text{Mg. of Standard}}{\text{Vol. Blood Filtrate Analyzed}} \times 1000 = W$$

$$\frac{19.7}{20} \times \frac{25}{25} \times \frac{0.4}{2} \times 1000 = 197$$

A glance at the left member of the equation shows that 19.7/20 is F, the variable factor. The balance of the left member of the equation is the constant factor C for the analytic procedure, and it equals 200. The significant figure in 200 is 2, and opposite 2 of the second column, under 19.7, the table shows 1.970. To get the correct value for 200, the decimal point must be moved two places to the right. This gives 197 checking the value found by calculation. The value of F, or 19.7/20, which is found to be 0.985, by calculation, can be verified by looking under 19.7, opposite 1 of the second column.

Values for Dividends Divided by 20 *and Multiplied by* 1 *to* 9 *When Colorimeter Readings Range from* 13.0 *to* 30.9[1]

D	C	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	1	0.650	0.655	0.660	0.665	0.670	0.675	0.680	0.685	0.690	0.695
	2	1.300	1.310	1.320	1.330	1.340	1.350	1.360	1.370	1.380	1.390
	3	1.950	1.965	1.980	1.995	2.010	2.025	2.040	2.055	2.070	2.085
	4	2.600	2.620	2.640	2.660	2.680	2.700	2.720	2.740	2.760	2.780
13	5	3.250	3.275	3.300	3.325	3.350	3.375	3.400	3.425	3.450	3.475
	6	3.900	3.930	3.960	3.990	4.020	4.050	4.080	4.110	4.140	4.170
	7	4.550	4.585	4.620	4.655	4.690	4.725	4.760	4.795	4.830	4.865
	8	5.200	5.240	5.280	5.320	5.360	5.400	5.440	5.480	5.520	5.560
	9	5.850	5.895	5.940	5.985	6.030	6.075	6.120	6.165	6.210	6.255
	1	0.700	0.705	0.710	0.715	0.720	0.725	0.730	0.735	0.740	0.745
	2	1.400	1.410	1.420	1.430	1.440	1.450	1.460	1.470	1.480	1.490
	3	2.100	2.115	2.130	2.145	2.160	2.175	2.190	2.205	2.220	2.235
	4	2.800	2820	2.840	2.860	2.880	2.900	2.920	2.940	2.960	2.980
14	5	3.500	3.525	3.550	3.575	3.600	3.625	3.650	3.675	3.700	3.725
	6	4.200	4.230	4.260	4.290	4.320	4.350	4.380	4.410	4.440	4.470
	7	4.900	4.935	4.970	5.005	5.040	5.075	5.110	5.145	5.180	5.215
	8	5.600	5.640	5.680	5.720	5.760	5.800	5.840	5.880	5.920	5.960
	9	6.300	6.345	6.390	6.435	6.480	6.525	6.570	6.615	6.660	6.705
	1	0.750	0.755	0.760	0.765	0.770	0.775	0.780	0.785	0.790	0.795
	2	1.500	1.510	1.520	1.530	1.540	1.550	1.560	1.570	1.580	1.590
	3	2.250	2.265	2.280	2.295	2.310	2.325	2.340	2.355	2.370	2.385
	4	3.000	3.020	3.040	3.060	3.080	3.100	3.120	3.140	3.160	3.180
15	5	3.750	3.775	3.800	3.825	3.850	3.875	3.900	3.925	3.850	3.975
	6	4.500	4.530	4.560	4.590	4.620	4.650	4.680	4.710	4.740	4.770
	7	5.250	5.285	5.320	5.355	5.390	5.425	5.460	5.495	5.530	5.565
	8	6.000	6.040	6.080	6.120	6.160	6.200	6.240	6.280	6.320	6.360
	9	6.750	6.795	6.840	6.885	6.930	6.975	7.020	7.065	7.110	7.155
	1	0.800	0.805	0.810	0.815	0.820	0.825	0.830	0.835	0.840	0.845
	2	1.600	1.610	1.620	1.630	1.640	1.650	1.660	1.670	1.680	1.690
	3	2.400	2.415	2.430	2.445	2.460	2.475	2.490	2.505	2.520	2.535
	4	3.200	3.220	3.240	3.260	3.280	3.300	3.320	3.340	3.360	3.380
16	5	4.000	4.025	4.050	4.075	4.100	4.125	4.150	4.175	4.200	4.225
	6	4.800	4.830	4.860	4.890	4.920	4.950	4.980	5.010	5.040	5.070
	7	5.600	5.635	5.670	5.705	5.740	5.775	5.810	5.845	5.880	5.915
	8	6.400	6.440	6.480	6.520	6.560	6.600	6.640	6.680	6.720	6.760
	9	7.200	7.245	7.290	7.335	7.380	7.425	7.470	7.515	7.560	7.605
	1	0.850	0.855	0.860	0.865	0.870	0.875	0.880	0.885	0.890	0.895
	2	1.700	1.710	1.720	1.730	1.740	1.750	1.760	1.770	1.780	1.790
	3	2.550	2.565	2.580	2.595	2.610	2.625	2.640	2.655	2.670	2.685
	4	3.400	3.420	3.440	3.460	3.480	3.500	3.520	3.540	3.560	3.580
17	5	4.250	4.275	4.300	4.325	4.350	4.375	4.400	4.425	4.450	4.475
	6	5.100	5.130	5.160	5.190	5.220	5.250	5.280	5.310	5.340	5.370
	7	5.950	5.985	6.020	6.055	6.090	6.125	6.160	6.195	6.230	6.265
	8	6.800	6.840	6.880	6.920	6.960	7.000	7.040	7.080	7.120	7.160
	9	7.650	7.695	7.740	7.785	7.830	7.875	7.920	7.965	8.010	8.055

Values for Dividends Divided by 20 *and Multiplied by* 1 *to* 9 *When Colorimeter Readings Range from* 13.0 *to* 30.9.[1]—*(Continued)*

D	C	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
18	1	0.900	0.905	0.910	0.915	0.920	0.925	0.930	0.935	0.940	0.945
	2	1.800	1.810	1.820	1.830	1.840	1.850	1.860	1.870	1.880	1.890
	3	2.700	2.715	2.730	2.745	2.760	2.775	2.790	2.805	2.820	2.835
	4	3.600	3.620	3.640	3.660	3.680	3.700	3.720	3.740	3.760	3.780
	5	4.500	4.525	4.550	4.575	4.600	4.625	4.650	4.675	4.700	4.725
	6	5.400	5.430	5.460	5.490	5.520	5.550	5.580	5.610	5.640	5.670
	7	6.300	6.335	6.370	6.405	6.440	6.475	6.510	6.545	6.580	6.615
	8	7.200	7.240	7.280	7.320	7.360	7.400	7.440	7.480	7.520	7.560
	9	8.100	8.145	8.190	8.235	8.280	8.325	8.370	8.415	8.460	8.505
19	1	0.950	0.955	0.960	0.965	0.970	0.975	0.980	0.985	0.990	0.995
	2	1.900	1.910	1.920	1.930	1.940	1.950	1.960	1.970	1.980	1.990
	3	2.850	2.865	2.880	2.895	2.910	2.925	2.940	2.955	2.970	2.985
	4	3.800	3.820	3.840	3.860	3.880	3.900	3.920	3.940	3.960	3.980
	5	4.750	4.775	4.800	4.825	4.850	4.875	4.900	4.925	4.950	4.975
	6	5.700	5.730	5.760	5.790	5.820	5.850	5.880	5.910	5.940	5.970
	7	6.650	6.685	6.720	6.755	6.790	6.825	6.860	6.895	6.930	6.965
	8	7.600	7.640	7.680	7.720	7.760	7.800	7.840	7.880	7.920	7.960
	9	8.550	8.595	8.640	8.685	8.730	8.775	8.820	8.865	8.910	8.955
20	1	1.000	1.005	1.010	1.015	1.020	1.025	1.030	1.035	1.040	1.045
	2	2.000	2.010	2.020	2.030	2.040	2.050	2.060	2.070	2.080	2.090
	3	3.000	3.015	3.030	3.045	3.060	3.075	3.090	3.105	3.120	3.135
	4	4.000	4.020	4.040	4.060	4.080	4.100	4.120	4.140	4.160	4.180
	5	5.000	5.025	5.050	5.075	5.100	5.125	5.150	5.175	5.200	5.225
	6	6.000	6.030	6.060	6.090	6.120	6.150	6.180	6.210	6.240	6.270
	7	7.000	7.035	7.070	7.105	7.140	7.175	7.210	7.245	7.280	7.315
	8	8.000	8.040	8.080	8.120	8.160	8.200	8.240	8.280	8.320	8.360
	9	9.000	9.045	9.090	9.135	9.180	9.225	9.270	9.315	9.360	9.405
21	1	1.050	1.055	1.060	1.065	1.070	1.075	1.080	1.085	1.090	1.095
	2	2.100	2.110	2.120	2.130	2.140	2.150	2.160	2.170	2.180	2.190
	3	3.150	3.165	3.180	3.195	3.210	3.225	3.240	3.255	3.270	3.285
	4	4.200	4.220	4.240	4.260	4.280	4.300	4.320	4.340	4.360	4.380
	5	5.250	5.275	5.300	5.325	5.350	5.375	5.400	5.425	5.450	5.475
	6	6.300	6.330	6.360	6.390	6.420	6.450	6.480	6.510	6.540	6.570
	7	7.350	7.385	7.420	7.455	7.490	7.525	7.560	7.595	7.630	7.665
	8	8.400	8.440	8.480	8.520	8.560	8.600	8.640	8.680	8.720	8.760
	9	9.450	9.495	9.540	9.585	9.630	9.675	9.720	9.765	9.810	9.855
22	1	1.100	1.105	1.110	1.115	1.120	1.125	1.130	1.135	1.140	1.145
	2	2.200	2.210	2.220	2.230	2.240	2.250	2.260	2.270	2.280	2.290
	3	3.300	3.315	3.330	3.345	3.360	3.375	3.390	3.405	3.420	3.435
	4	4.400	4.420	4.440	4.460	4.480	4.500	4.520	4.540	4.560	4.580
	5	5.500	5.525	5.550	5.575	5.600	5.625	5.650	5.675	5.700	5.725
	6	6.600	6.630	6.660	6.690	6.720	6.750	6.780	6.810	6.840	6.870
	7	7.700	7.735	7.770	7.805	7.840	7.875	7.910	7.945	7.980	8.015
	8	8.800	8.840	8.880	8.920	8.960	9.000	9.040	9.080	9.120	9.160
	9	9.900	9.945	9.990	10.035	10.080	10.125	10.170	10.215	10.260	10.305
23	1	1.150	1.155	1.160	1.165	1.170	1.175	1.180	1.185	1.190	1.195
	2	2.300	2.310	2.320	2.330	2.340	2.350	2.360	2.370	2.380	2.390
	3	3.450	3.465	3.480	3.495	3.510	3.525	3.540	3.555	3.570	3.585
	4	4.600	4.620	4.640	4.660	4.680	4.700	4.720	4.740	4.760	4.780
	5	5.750	5.775	5.800	5.825	5.850	5.875	5.900	5.925	5.950	5.975
	6	6.900	6.930	6.960	6.990	7.020	7.050	7.080	7.110	7.140	7.170
	7	8.050	8.085	8.120	8.155	8.190	8.225	8.260	8.295	8.330	8.365
	8	9.200	9.240	9.280	9.320	9.360	9.400	9.440	9.480	9.520	9.560
	9	10.350	10.395	10.440	10.485	10.530	10.575	10.620	10.665	10.710	10.755
24	1	1.200	1.205	1.210	1.215	1.220	1.225	1.230	1.235	1.240	1.245
	2	2.400	2.410	2.420	2.430	2.440	2.450	2.460	2.470	2.480	2.490
	3	3.600	3.615	3.630	3.645	3.660	3.675	3.690	3.705	3.720	3.735
	4	4.800	4.820	4.840	4.860	4.880	4.900	4.920	4.940	4.960	4.980
	5	6.000	6.025	6.050	6.075	6.100	6.125	6.150	6.175	6.200	6.225
	6	7.200	7.230	7.260	7.290	7.320	7.350	7.380	7.410	7.440	7.470
	7	8.400	8.435	8.470	8.505	8.540	8.575	8.610	8.645	8.680	8.715
	8	9.600	9.640	9.680	9.720	9.760	9.800	9.840	9.880	9.920	9.960
	9	10.800	10.845	10.890	10.935	10.980	11.025	11.070	11.115	11.160	11.205
25	1	1.250	1.255	1.260	1.265	1.270	1.275	1.280	1.285	1.390	1.295
	2	2.500	2.510	2.520	2.530	2.540	2.550	2.560	2.570	2.580	2.590
	3	3.750	3.765	3.780	3.795	3.810	3.825	3.840	3.855	3.870	3.885
	4	5.000	5.020	5.040	5.060	5.080	5.100	5.120	5.140	5.160	5.180
	5	6.250	6.275	6.300	6.325	6.350	6.375	6.400	6.425	6.450	6.475
	6	7.500	7.530	7.560	7.590	7.620	7.650	7.680	7.710	7.740	7.770
	7	8.750	8.785	8.820	8.855	8.890	8.925	8.960	8.995	9.030	9.065
	8	10.000	10.040	10.080	10.120	10.160	10.200	10.240	10.280	10.320	10.360
	9	11.250	11.295	11.340	11.385	11.430	11.475	11.520	11.565	11.610	11.655

[1] The integral parts of the dividends, D, are shown in the first column and the fractional parts at the top of the page. The constant factors, C, are shown in the second column, and products are opposite them in each case, under the appropriate fractional part of the dividend. Values from 0.001 to 1000 times as great are obtained by moving the decimal point. After McCrakan, R. F., et al., Arch. Path. and Lab. Med., vol. 3, page 227, Feb. 1927.

Values for Dividends Divided by 20 *and Multiplied by* 1 *to* 9 *when Colorimeter Readings Range from* 13.0 *to* 30.9.[1]—(*Concluded*)

D	C	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	1	1.300	1.305	1.310	1.315	1.320	1.325	1.330	1.335	1.340	1.345
	2	2.600	2.610	2.620	2.630	2.640	2.650	2.660	2.670	2.680	2.690
	3	3.900	3.915	3.930	3.945	3.960	3.975	3.990	4.005	4.020	4.035
	4	5.200	5.220	5.240	5.260	5.280	5.300	5.320	5.340	5.360	5.380
26	5	6.500	6.525	6.550	6.575	6.600	6.625	6.650	6.675	6.700	6.725
	6	7.800	7.830	7.860	7.890	7.920	7.950	7.980	8.010	8.040	8.070
	7	9.100	9.135	9.170	9.205	9.240	9.275	9.310	9.345	9.380	9.415
	8	10.400	10.440	10.480	10.520	10.560	10.600	10.640	10.680	10.720	10.760
	9	11.700	11.745	11.790	11.835	11.880	11.925	11.970	12.015	12.060	12.105
	1	1.350	1.355	1.360	1.365	1.370	1.375	1.380	1.385	1.390	1.395
	2	2.700	2.710	2.720	2.730	2.740	2.750	2.760	2.770	2.780	2.790
	3	4.050	4.065	4.080	4.095	4.110	4.125	4.140	4.155	4.170	4.185
	4	5.400	5.420	5.440	5.460	5.480	5.500	5.520	5.540	5.560	5.580
27	5	6.750	6.775	6.800	6.825	6.850	6.875	6.900	6.925	6.950	6.975
	6	8.100	8.130	8.160	8.190	8.220	8.250	8.280	8.310	8.340	8.370
	7	9.450	9.485	9.520	9.555	9.590	9.625	9.660	9.695	9.730	9.765
	8	10.800	10.840	10.880	10.920	10.960	11.000	11.040	11.080	11.120	11.160
	9	12.150	12.195	12.240	12.285	12.330	12.375	12.420	12.465	12.510	12.555
	1	1.400	1.405	1.410	1.415	1.420	1.425	1.430	1.435	1.440	1.445
	2	2.800	2.810	2.820	2.830	2.840	2.850	2.860	2.870	2.880	2.890
	3	4.200	4.215	4.230	4.245	4.260	4.275	4.290	4.305	4.320	4.335
	4	5.600	5.620	5.640	5.660	5.680	5.700	5.720	5.740	5.760	5.780
28	5	7.000	7.025	7.050	7.075	7.100	7.125	7.150	7.175	7.200	7.225
	6	8.400	8.430	8.460	8.490	8.520	8.550	8.580	8.610	8.640	8.670
	7	9.800	9.835	9.870	9.905	9.940	9.975	10.010	10.045	10.080	10.115
	8	11.200	11.240	11.280	11.320	11.360	11.400	11.440	11.480	11.520	11.560
	9	12.600	12.645	12.690	12.735	12.780	12.825	12.870	12.915	12.960	13.005
	1	1.450	1.455	1.460	1.465	1.470	1.475	1.480	1.485	1.490	1.495
	2	2.900	2.910	2.920	2.930	2.940	2.950	2.960	2.970	2.980	2.990
	3	4.350	4.365	4.380	4.395	4.410	4.425	4.440	4.455	4.470	4.485
	4	5.800	5.820	5.840	5.860	5.880	5.900	5.920	5.940	5.960	5.980
29	5	7.250	7.275	7.300	7.325	7.350	7.375	7.400	7.425	7.450	7.475
	6	8.700	8.730	8.760	8.790	8.820	8.850	8.880	8.910	8.940	8.970
	7	10.150	10.185	10.220	10.255	10.290	10.325	10.360	10.395	10.430	10.465
	8	11.600	11.640	11.680	11.720	11.760	11.800	11.840	11.880	11.920	11.960
	9	13.050	13.095	13.140	13.185	13.230	13.275	13.320	13.365	13.410	13.455
	1	1.500	1.505	1.510	1.515	1.520	1.525	1.530	1.535	1.540	1.545
	2	3.000	3.010	3.020	3.030	3.040	3.050	3.060	3.070	3.080	3.090
	3	4.500	4.515	4.530	4.545	4.560	4.575	4.590	4.605	4.620	4.635
	4	6.000	6.020	6.040	6.060	6.080	6.100	6.120	6.140	6.160	6.180
30	5	7.500	7.525	7.550	7.575	7.600	7.625	7.650	7.675	7.700	7.725
	6	9.000	9.030	9.060	9.090	9.120	9.150	9.180	9.210	9.240	9.270
	7	10.500	10.535	10.570	10.605	10.640	10.675	10.710	10.745	10.780	10.815
	8	12.000	12.040	12.080	12.120	12.160	12.200	12.240	12.280	12.320	12.360
	9	13.500	13.545	13.590	13.635	13.680	13.725	13.770	13.815	13.860	13.905

Congo-red Paper.—Dissolve 2 gs. Congo-red powder in enough water to make a liter of solution. Pass strips of filter-paper through the solution and hang the strips up to dry in an atmosphere free from chemical fumes. Such a place is a well-ventilated hood, capable of being isolated from other hoods used for current work.

Cyanid of Potassium.—Fatal doses: Mammals: Rabbit about 2 mgs. per kilo. Man, 5 drops of a 2 per cent. solution. Consequently, 3 drops of the solution used in blood analysis. Treatment: Stomach lavage by large tube; emetic; permanganate, 0.5 per cent. solution in water; artificial respiration; rest.

Dialyzing Membranes.—See *Collodion.*

Di-azo-benzene-sulphonic Acid.—See Ehrlich, below.

Dichromate, Potassium.—See page 922.

Ehrlich's Di-azo Reagent.—(1) For indol: Para-dimethyl-amino-benzaldehyde 4 gms., ethanol 380 mls., HCl (conc.) 80 mls. Use 1 volume of the fluid to be tested and one of the reagent, a positive color being red. (2) (Used in diagnosis of typhoid.) Reagents: Sulphanilic acid:

Saturated 5 per cent. HCl solution; $NaNO_2$, 0.5 per cent.; NH_4OH conc. A froth appears on shaking the reagent when mixed with the unknown solution; positive test gives a pink froth.

Epinephrin (adrenalin; suprenin) for collapse in experimental animal: Mammals, intravenous, 0.25 ml. of a 1:5000 solution per kilo.

To induce glycosuria in rabbit: 2 mls. of 1:1000 solution hypodermically for an average 2-kilogram rabbit.

Esbach's Reagent.—Dissolve 10 gs. picric acid in 600 mls. of distilled water. Add 10 gs. citric acid. After complete dissolution, add water to make a total volume of 1000 mls.

Ethanol.—Table for diluting different strengths of ethanol to obtain other strengths: The figures in the table under the different percentages are the number of mls. which have to be added to 100 mls. of the alcohol indicated in the top line in percents. to give a diluted alcohol of the strength indicated in the column to the left. That is, if one has 85 per cent. alcohol and wishes 50 per cent., 74 mls. of distilled water must be added to every 100 mls. of the stronger solution to make the weaker:

To make ethanol solutions from:

To:	95 per cent. Add water:	90 per cent.	85 per cent.	80 per cent.	75 per cent.	70 per cent.	65 per cent.	60 per cent.	55 per cent.	50 per cent.
85	13	6								
80	21	14	7							
75	29	22	14	7						
70	40	31	23	15	8					
65	49	42	33	25	16	8				
60	62	54	44	35	26	18	9			
55	75	68	58	48	38	29	19	9		
50	95	85	74	63	52	42	31	20	10	
45	117	105	94	81	70	58	46	34	23	11
40	152	130	117	104	91	78	65	51	38	26
35	196	163	148	133	118	103	88	70	58	44
30	222	206	189	171	154	136	119	102	85	67
25	287	266	245	224	204	183	162	142	121	101
20	382	356	330	304	278	253	227	201	176	151
15	539	505	471	437	403	369	335	301	267	234
10	856	805	754	703	652	602	551	500	450	400

Ethanol contaminated with diethyl phthalate: Transfer about 10 mls. of the mixture of ethanol and phthalate to a small evaporating dish and evaporate rapidly over a bunsen flame. When almost dry, mix the residue with about 1 g. boric acid. Continue heating to dryness. To the cake add about 1 g. resorcinol. Continue heating; a sticky mass results. Cool. Add about 50 mls. H_2O, transfer to a 200 × 20-mm. test tube or 50 ml. volumetric cylinder. Neutralize with 10 per cent. NaOH solution and make slightly alkaline. A positive test for phthalate is indicated by the appearance of fluorescence.

Fehling's Sugar Reagents.—Three solutions, quantitative: (1) Dissolve 103.92 gs. cupric sulphate, $CuSO_4.5H_2O$, in warm (60° C.) distilled water and dilute to 1000 mls. with water.

(2) Dissolve 320 gs. potassium sodium tartrate (Rochelle salt) in warm water and add a little phenol to prevent plant growth. Dilute to 1 liter with water.

(3) Dissolve 150 gs. sodium hydroxid in distilled water and dilute to 1 liter.

In use, mix equal quantities of (1), (2), and (3). Ten mls. of the mixed solution = 0.05 g. glucose, 0.0511 g. galactose, 0.0431 g. mannose, or 0.05144 g. fructose.

Ferric Chlorid Normal Solution.—Dissolve 54 gs. Fe_2Cl_6 in enough cold water to make 1000 mls. of fluid.

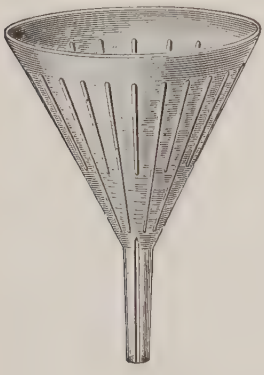


Fig. 267.—"Physiological" or fluted filter. The ribbing prevents too close application of the paper to the sides of the funnel.[1]

Fig. 268.—Another form of fluted or "physiological" funnel. Compare Fig. 267.

Fig. 269.—Alundum crucible which is frequently used in place of a Gooch crucible for filtering purposes. See Fig. 270.

Fig. 270.—The use of the crucible in filtering. A Gooch crucible or, better, in most cases, an alundum crucible is applied to the "carbon" or filter-tube by means of a piece of wide, thin rubber tubing, R. The carbon tube passes through a stopper into the filter-flask, F.

Fibrin-carmin.—Dissolve 1 g. of carmin in 1 ml. of ammonium hydroxid. Add about 400 mls. of distilled water. Leave in a flask or bottle stoppered with cotton wool until the odor of ammonia has nearly gone. Into this solution drop finely chopped, thoroughly washed fibrin and leave for twenty-four hours. Then strain the fibrin from the fluid, wash in running water until the washings are colorless, and store in

[1] A small (25-ml. or smaller) funnel also is often called a "physiological funnel."

ethyl ether in a well-stoppered flask or bottle. When required for use, wash the fibrin free from ether. Method of Cole.

Folin's Uric Acid Reagent (from $Na_2WO_4.2H_2O$, J. T. Baker Co. Special).—Add to 160 mls. H_2O, 50 mls. syrupy H_3PO_4. Heat to 85° C. and add 100 gs. $Na_2WO_4.2H_2O$. Boil gently for one hour beneath a condenser. In a liter beaker place 25 gs. Li_2CO_3, 50 mls. H_3PO_4, and 200 mls. H_2O. Boil ten minutes, cool, and add to the first solution. Mix and dilute with water to 1000 mls.

Folin-Denis Uric-acid Reagent.—To 750 gs. of distilled water add 100 gs. of sodium tungstate and 80 mls. of 85 per cent. syrupy phosphoric acid. Boil gently for two hours under a Hopkins reflux condenser. Cool and transfer to a 1000-ml. volumetric flask. Make up to the mark with distilled water. Check against a small amount of uric acid. Only nitrate-free reagents may be used. If the solution is very dark, add a few drops of bromin and boil fifteen minutes.

Folin-Trimble Modification.—Heat together 10 gs. Li_2CO_3, 20 of H_3PO_4, 80 of H_2O. Add 80 gs. $P_2O_5.24WO_3.xH_2O$. Boil one hour. Add 15 gs. Li_2CO_3 + 65 gs. H_3PO_4 + 200 H_2O. Mix the two solutions and dilute to 1 liter.

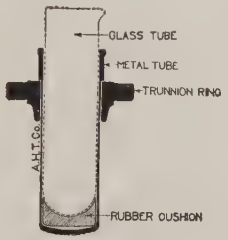


Fig. 271.—Fifty-milliliter centrifuge tube (outlined) with its proper situation in the centrifuge cup.

Folin-Denis Phenol Reagent.—To 750 mls. of distilled water add 100 gs. of sodium tungstate. Dissolve. Then add 20 gs. phosphomolybdic acid and finally 50 mls. of syrupy 85 per cent. phosphoric acid. Boil for two hours under reflux condenser, cool, and dilute to 1000 mls. Check against a diluted solution of phenol. The tungstate must contain no molybdate. For method of detecting the presence of molybdenum in tungstate mixtures see Jour. Biol. Chem., vol. 60, page 474, 1924.

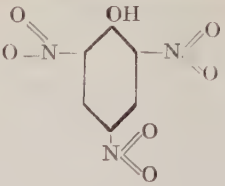
Folin-Wu Silver Lactate (Lactic Acid Solution).—Into a 100-ml. volumetric flask place 5 gs. silver lactate, $H_3C.CHOH.COO.Ag.H_2O$,[1] and 5 gs. lactic acid (specific gravity 1.21; 85 per cent. $C_3H_6O_3$); dilute to 100 mls. with water.

Folin-McEllroy Sugar Reagents.—Qualitative: Dissolve 100 gs. sodium pyrophosphate, $Na_4P_2O_7.10H_2O$, 30 gs. crystal di-sodium-monohydrogen phosphate, Na_2HPO_4, and 50 gs. of dry sodium carbonate, Na_2CO_3, in about 900 mls. of distilled water. Add, after these substances have been dissolved, a solution of 13 gs. cupric sulphate dissolved in 200 mls. of distilled water. The solution is ready for use.

Quantitative: (1) Acidified cupric sulphate solution: Weigh 60 gs. of highest purity $CuSO_4.5H_2O$ crystals and make up to about 900 mls. After the crystals have dissolved add 5 mls. of concentrated sulphuric acid and make up to 1000 mls. with distilled water.

(2) Phosphate-carbonate-thiocyanate dry mixture: Mix 100 gs. dry di-sodium monohydrogen phosphate, $Na_2HPO_4.12H_2O$, 60 gs. monohydrated sodium carbonate, $Na_2CO_3.H_2O$, and 30 gs. of potassium thiocyanate, KCNS. Mix in a large mortar, leave overnight protected by a large filter-paper, then grind again, and bottle in a salt-mouth bottle (Fig. 260).

[1] The Powers-Weightman-Rosengarten Co. preparation contains 8.4 per cent. H_2O and 50.2 per cent. silver.

Folin-McEllroy Lactose Reagents.—(1) Saturated picric acid solution, aqueous; weigh roughly 2 gs. of trinitrophenol,  and dissolve in lukewarm distilled water; make up to 100 mls. with water.

(2) Twenty per cent. sodium carbonate solution: Weigh 20 gs. dry sodium carbonate (Na_2CO_3) and make up to 100 mls. with distilled water. If made from $Na_2CO_3.10H_2O$ crystals, use 5.5 gs.

Folin's Standard Creatinin Solution from Creatinin-zinc-chlorid.—Weigh exactly 1.6106 gs. of creatinin zinc chlorid, and dissolve in 1000 mls. of decinormal hydrochloric acid. Each ml. contains 1.0 mg. of creatinin. For creatin, weigh 1.389 g. creatinin-zinc-chlorid and dilute to 1000 mls. with distilled water. Each ml. contains the equivalent of 1.0 mg.

For blood creatinin make the following standard: Into a liter volumetric flask pipette 6 mls. of the above solution; add 10 mls. normal HCl solution, 5 drops toluene, and dilute to the mark. 5 mls. = 0.03 mg. creatinin.

Folin's Uric Acid-formaldehyde Standard Solution.—Weigh exactly 1 g. of uric acid crystals and transfer them to a small funnel in a liter volumetric flask. Wash the crystals through into the flask by means of the following solution: Into a 300-ml. beaker place about 0.5 gram of Li_2CO_3 powder and add 150 mls. of distilled water. Heat to about 60° C. and stir until all the powder has dissolved. Use this hot solution for rinsing the funnel. When the uric acid crystals have dissolved, cool the flask under the tap and add about 400 mls. of water. Add 25 mls. of commercial "formalin"[1] solution and 3 mls. of glacial acetic acid. Agitate the contents of the flask to remove the CO_2 gas, dilute to 1000 mls., and mix. Keep the stock solution in small bottles (about 100 mls.) filled completely to avoid contact with air. Each ml. of this stock solution contains 1 mg. of uric acid. For use: Dilute 5 mls. of the stock solution to 50 mls. with water; each 10 mls. then contain 1 mg. of uric acid.

Folin's Special Sulphuric Acid for Protid Precipitation, Two-thirds Normal.—Dilute 36 mls. concentrated H_2SO_4 with water to make 1000 mls. of fluid.

Folin's Special Lithium Oxalate for Anticoagulant Action.—To 50 gs. Li_2CO_3 add 85 gs. oxalic acid. To this mixture add 1 liter of water at 80° C. Stir slowly; CO_2 will be evolved. After complete solution pour the solution into a large evaporating dish and distill off the water to a dry residue. Powder the residue in a mortar. This preparation is twice as potent as an anticoagulant as K-oxalate and dissolves much more quickly. It causes little precipitation with other reagents used in blood analysis. Lithium oxalate may be used as oxalated cloth: Cut strips of "birdseye" cloth, starch free 10 cms. long and 10 cms. wide. Into a liter beaker place 10 gs. lithium carbonate and 17 gs. oxalic acid. Add 240 mls. of distilled water at 75° C. While the solution is still warm pour it into a shallow dish (large evaporating dish) and pass strips of the cotton material through it, as one develops a camera film. Hang the strips in a hood free from fumes. The cloth contains 20 per cent. lithium oxalate. Fifty mgs. of the cloth thus treated will suffice to prevent clotting in from 15 to 20 mls. of blood.

Folin's Lithium Sulphate Solution.—Dissolve 20 gs. of powdered lithium sulphate, $Li_2SO_4.H_2O$, in 80 mls. of cold water. Dilute to 100 mls. with water and, after standing over night, filter.

[1] A 40 per cent. aqueous solution of H.CHO gas.

Folin's Special Sodium Cyanid Solution.—Weigh 15 gs. of sodium cyanid, NaCN, into a 100-ml. volumetric flask and add enough 0.1 normal solution of sodium hydroxid to make 100 mls. Use a pure white NaCN for this work (NaCN is decomposed by CO_2 from the air). It is best to let the solution stand for some time, and to this purpose make the following stock solution to be kept three months: Weigh 100 gs. of NaCN into a beaker and add 670 mls. of 0.1 normal sodium hydroxid solution. Leave the beaker exposed to the air to prevent the formation of brownish azulmic acid, $C_4H_5N_5O$.[1]

Folin's Solutions for Amino-acids.—(1) Standard glycin solution containing 0.07 mg. nitrogen per ml.: Weigh 0.3752 g. pure glycin, CH_2NH_2COOH, into a 100-ml. volumetric flask and add 200 mgs. of sodium benzoate,

Make up to the mark with 0.1 normal HCl solution.

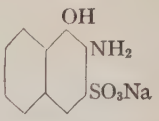
(2) Special sodium carbonate solution: This is a carbonate-bicarbonate solution of about 1 per cent. concentration: Saturate, at room temperature, about 75 mls. of distilled water with sodium carbonate 25 gs. of dry Na_2CO_3, or 75 gs. of crystal, $Na_2CO_3.10H_2O$. Pour 50 mls. of this solution into a 500-ml. volumetric flask and make up to the mark. Titrate 20 mls. of this solution against 0.1 normal HCl solution, methyl red, indicator. Using the figure obtained in this titration, dilute the carbonate solution until 8.5 mls. neutralize 20 mls. of the decinormal acid. When 1 ml. of this properly diluted solution is added to 1 ml. of the amino-acid preparation described in (1), the alkalinity of the mixture is correct for the development of color with the following reagent.

(3) Beta-naphthoquinon sulphonic acid, 0.5 per cent. solution. Preparation of the reagent: (*β*) Transfer 100 gs. of *a*-naphthol to a liter beaker; add 300 mls. of a 10 per cent. NaOH solution and stir until dissolved (fifteen minutes). (*b*) Transfer 50 to 55 gs. of $NaNO_2$ to a large (4000-ml.) beaker; add about 600 mls. of distilled water until complete solution. (*c*) Pour (*a*) into the beaker and rinse with about 100 mls. H_2O. (*d*) Add 800 gs. crushed, clean ice. (*e*) Fill a 200-ml. volumetric cylinder with cold 10 per cent. H_2SO_4 solution; empty, slowly, the cylinder into the 4-liter beaker, pouring the contents down the side, stirring vigorously, and continue the stirring for a few minutes after apparent solution has occurred. Fill the cylinder again to the 200-ml. mark with the dilute acid and repeat. Continue the procedure until 800 mls. of the dilute acid have been added. Dip a piece of Congo-red indicator paper into the solution in the large beaker; the reaction should be sufficiently acid to turn the color blue. About fifteen to twenty minutes should have been consumed by this time. The yellow, semisolid paste which has formed should be just tinged with green; a distinct greenish color signifies that the conditions are not right for a good yield. Leave for one hour or longer. (*f*) Using a Buchner funnel and aspirating pump, filter off the precipitate and wash with a liter and a half of cold water. Save the residue. (*g*) Transfer the precipitate (nitroso-beta-naphthol) to a large evaporating dish; add, by sprinkling, 100 gs. of sodium bisulphite, $NaHSO_3$, and 50 gs. of sodium sulphite, Na_2SO_3. Stir with enamel or porcelain spoon; the solution becomes liquid. Filter on a Buchner funnel through a double layer of quantitative filter-paper. The residue will be small. Wash with a little cold water. (*h*) Immediately transfer the filtrate to a large (5-liter) amber bottle containing 2 liters of distilled water and 500 mls. of concentrated HCl. Cover with a funnel and place a watch-glass in the mouth. Leave the preparation in a dark closet for about two days. If

[1] NaCN solutions give, on standing, such products as urea, ammonium cyanate, formate, oxalate, etc. In alkaline solution this condensation does not occur.

light has been excluded there will be found a mass of light needle crystals. Filter by means of a Buchner funnel and wash the residue with 2 liters of cold water.

The residue is the Na salt of amino-naphthol-sulphonic acid,



(*i*)

Transfer the precipitate to a large beaker (4 liters). Sprinkle over the precipitate 100 gs. $NaNO_3$. Add a solution of 100 mls. of concentrated HNO_3 diluted with 350 mls. of distilled water to the beaker. Place the preparation in a hood because fumes of nitric acid arise. Leave for ten minutes, then stir, and then let stand half an hour. If no reaction take place, add from 1 to 5 mls. of concentrated HNO_3. Filter after the half-hour and wash with a liter of 10 NaCl solution. Now purify this product, sodium naphthoquinon sulphonate: (*j*) Transfer the substance to a large porcelain dish. Add 200 gs. powdered borax and 450 mls. of H_2O. Mix. Filter through paper (Buchner funnel) to remove the extra borax and the black impurity; wash with about 150 mls. of cold distilled water, combining washings with the filtrate and transfer to a 4-liter beaker. Add a few drops of liquid bromin to a solution of 850 mls. of 95 per cent. ethanol and 150 mls. of concentrated HCl, after the acid alcohol solution has been cooled under the tap. Agitate the solution until the bromin has been completely dissolved and pour the solution into the filtrate in the 4-liter beaker. Mix rapidly with stirring-rod. Leave five minutes; the quinon should have been nearly completely precipitated at this time. Filter on a Buchner funnel, wash with 700 to 800 mls. of 10 per cent. NaCl. Repeat once in order to remove ammonia: Omit the washing with NaCl solution and in its place use first 300 to 400 mls. of ethanol and then 200 mls. of ethyl ether. Yield: About 80 gs. of the beta-naphthoquinon-sulphonic acid. Tests: A 1 per cent. solution in water should yield a color which, compared with a half-normal potassium dichromate solution (page 736) is as bichromate 20 mms. on the colorimeter, quinon 26 to 27 mms. Two mls. of the 1 per cent. solution mentioned above should become bleached when diluted with 25 mls. of water, treated with 1 ml. of 50 per cent. acetic acid solution, and finally with 1 ml. of a 15 per cent. sodium thiosulphate solution. The absence of ammonia may be demonstrated by adding 2 gs. of "permutit" powder (page 715) to 10 mls. of the 1 per cent. solution of the quinon, shaking for about five minutes, and then decant as in the ammonia determination; finally, adding 10 per cent. NaOH solution to the powder residue and 5 mls. of Nessler's solution (see below). There should be no yellow color.

Preparation of the working solution: Weigh, roughly on a torsion balance, 0.1 g. of the dry beta-naphthoquinon-sulphonic acid into a small flask, add 20 mls. of distilled water, and mix.

(4) Special acetic-acetate solution: Dilute 50 mls. of glacial acetic acid with 100 mls. of 5 per cent. sodium acetate solution. The acetate intensifies the color of the preparation of the quinon with amino-acid and retards the formation of turbidity due to the sulphur of the thiosulphate.

(5) Four per cent. solution of sodium thiosulphate, $Na_2S_2O_3.5H_2O$: Weigh 4 gs. of the pea-sized crystals and dilute with water to 100 mls.

Folin's Modification of Nessler-Winkler Reagent.—Transfer 150 gs. of KI and 110 gs. of resublimed iodin to a 500-ml. Florence flask. Add 100 mls. of H_2O and about 150 gs. of mercury. Shake the flask vigorously, preferably in a shaking machine, for fifteen minutes, or at least until the flakes of iodin have dissolved. The solution becomes hot. When the reddish iodin solution has become greenish owing to the formation of the double iodid, $HgI_2.2KI$, cool the flask in running tap-water. Decant the supernatant fluid from the residue of mercury followed by washing the residue

with water. Dilute to 2 liters. The solution should be clear; if it is not, it was permitted to stand too long before cooling. To complete the solution make the following solution of sodium hydroxid: Weigh on the laboratory scales 550 gs. of sodium hydroxid sticks by alcohol and dissolve them in about 800 mls. of distilled water by the aid of a stirring-rod. When solution is complete, let the preparation cool to about 50° C. and then continue the cooling by placing the flask in a vessel under the cold-water tap. When the solution reaches a temperature of 20° C., pour it into a liter cylinder and make up to the mark with distilled water. Mix and let stand for a night or longer. Then decant the clear, supernatant fluid from the residue and determine its normality by titrating 1 ml. against standard acid. With this data dilute the decanted fluid so that it makes a 10 per cent. solution. A normal solution of NaOH is 4 per cent., that is, 4 gs. in 100 mls. of solution; there is required 10 gs. in 100 mls. of solution. Making the completed Nessler's solution: Pour 350 mls. of the 10 per cent. NaOH solution just described into a 500-ml. volumetric flask and add 75 mls. of the double iodid solution first made; add distilled water to the mark (500 mls.). Retain the double iodid and the 55 per cent. NaOH solutions as stock solutions from which the finished Nessler reagent may be made when needed.

Folin's Standard Nitrogen Solution.—Preparation of pure ammonium sulphate crystals: Using a good commercial grade of ammonium sulphate crystals, in a flask dissolve 165 gs. in about 300 mls. of distilled water and then add 100 gs. of pure NaOH sticks. Immediately connect the flask with a Liebig condenser and collect the ammonia evolved in about 275 mls. of concentrated, freshly opened sulphuric acid. Distill by the aid of heat. $(NH_4)_2SO_4$ is produced in the acid and may be purified by adding 1 volume of ethanol, 95 per cent. Dry the crystals to constant weight in a desiccator over H_2SO_4 (page 93). For the standard, weigh 0.4716 g. of the crystals, dissolve in a small amount of distilled water, and make up to 1000 mls.; each 10 mls. contain 1 mg. nitrogen.

Folin's Special Urease Solution.—Transfer about 3 gs. of special preparation of "permutit" (page 715) for blood and urine chemistry, to a flask and add about 50 mls. of 2 per cent. acetic acid. Twirl the flask in order to thoroughly mix the powder with the acid solution, then let the flask rest, inclined toward one side and decant as much as possible of the supernatant liquid from the powder. Repeat, using distilled water, and repeat still again, using more distilled water. Add about 5 gs. of soy-bean or jack-bean meal to the powder after the last decantation and follow this with 100 mls. of 15 per cent. ethanol (see table of dilution, page 907). Shake gently, but continuously for ten minutes, filter through paper, saving the filtrate. Keep in an ice-box for not over four weeks.

Folin's Buffer Mixtures for Urease Action.—(1) Dissolve 69 gs. of $NaH_2PO_4.H_2O$ and 179 gs. of $Na_2HPO_4.12H_2O$ in 800 mls. H_2O. Cool and dilute to 1000 mls. Add 2 mls. of toluene, shake well, and keep in a well-stoppered flask.

(2) Dissolve 14 gs. of sodium pyrophosphate, $Na_4P_2O_7.10H_2O$, in enough of the following solution to make 100 mls.: Add 20 mls. of the 85 per cent. syrupy phosphoric acid to a liter flask and make up to the mark. Determine the normality of this solution by titrating 5 mls. against 0.1 normal sodium hydroxid and dilute to make the solution 0.5 normal.

Folin's Creatinin Reagent.—Add 75 mls. of 10 per cent. NaOH solution (see top of page under Folin's Nessler-Winkler solution) to a liter volumetric flask and dilute to the mark with a saturated (2 per cent.) picric acid solution.

Folin's Special Cupric Sulphate Solution for Lactose Determinations.—Weigh 6.05 gs. of pure $CuSO_4.5H_2O$ into a 100-ml. volumetric flask and add water enough to cause solution by the aid of a hot-water bath. Then dilute to the mark.

Folin's Standard Stock Solution for Quantitative Aceton Determinations.—Method using commercial aceton-sulphite:[1] Transfer 2.5 gs. of the aceton-sulphite powder to a liter volumetric flask; wash it with about 50 mls. of distilled water. Dilute concentrated hydrochloric acid 1:5 and make up the solution in the liter flask to the mark with this acid solution. Mix. Transfer 25 mls. of this solution to a 150-ml. Florence flask; add 20 mls. of decinormal iodin solution (page 918), let stand five minutes. Titrate the excess iodin with decinormal thiosulphate solution (page 926). This titration gives the sodium bisulphite content of the solution of sulphite-aceton. Now determine the aceton by difference: To another 25-ml. volume of the sulphite-aceton solution in the liter flask add 50 mls. decinormal iodin, let stand as before, and then add 10 mls. 10 per cent. NaOH solution. Leave five minutes and then add 18 mls. of concentrated HCl. Titrate as before with thiosulphate. From the amount of iodin used, 50 mls., subtract the burette reading just made, and from the remainder, the figure representing the iodin corresponding to the bisulphite content. You have now standardized the sulphite-aceton solution in the liter flask. From this figure make a solution containing 0.5 mg. of aceton per 10 mls. of fluid, using the standardized bisulphite-aceton solution.

Folin's Special Acid Mixture for Nitrogen Determinations.—(1) Dissolve 5 gs. of pure $CuSO_4.5H_2O$ crystals in about 50 mls. of distilled water by the aid of a water-bath and make up to 100 mls. with distilled water after cooling to room temperature. (2) Pour this solution into a liter flask and add 600 mls. of 85 per cent. syrupy phosphoric acid. Mix. (3) To this mixture add 200 mls. of ammonia-free concentrated sulphuric acid. Keep well stoppered.

Folin's Reagents for the Folin Modification of Benedict's Method for Uric Acid in the Blood.—(1) Cyanide solution: Weigh, with great care, 15 gs. of lump sodium cyanid, NaCN, into a beaker. Dissolve in about 50 mls. of decinormal NaOH. Transfer the solution to a 100-ml. volumetric flask and make up to the mark with the NaOH solution.

(2) Uric acid reagent; see Folin-Denis solution, page 909.

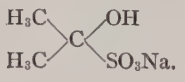
(3) Uric acid standard solution in formalin; see page 910.

(4) Lithium sulphate solution; see page 910.

Folin-Wu Reagents for Quantitative Determination of Blood-sugar.—(1) Standard sugar solution. (*a*) Benzoate solution: Dissolve 2.5 gs. of benzoic acid, , in about 500 mls. of boiling water in a beaker. In another beaker boil about a liter of water. Transfer the benzoate solution to a liter volumetric flask while the solution is hot, by means of a funnel and wash the solution adhering to the funnel into the flask with enough hot water to make a liter of solution. Cool and make to the mark with cold distilled water.

(*b*) Weigh exactly 1 gram of highest purity glucose powder and transfer it to a small funnel in the mouth of a 100-ml. volumetric flask. Wash the powder into the flask with about 50 mls. of the benzoate solution (1). Mix thoroughly and dilute to the mark with the benzoate solution. From this stock solution make as desired the working standard of a strength suited to the concentration of sugar in the

[1] This is a commercial preparation used in photography. It is obtainable through dealers in such supplies, or on order from the Eastman Kodak Company, Rochester, N. Y. The formula is:



unknown: (A) Weaker standard: Pipette 1 ml. of the stock solution (*b*) into a 100-ml. volumetric flask and dilute to the mark with the benzoate solution (1); 1 ml. of this standard contains 0.1 ml. of glucose. (B) Stronger standard: Pipette 2 mls. of the stock solution (*b*) into a 100-ml. volumetric flask and dilute to the mark with the benzoate solution (1); 2 mls. contain 0.4 mg. of glucose.

(2) Alkaline cupric sulphate solution: Dissolve 40 gs. of dry sodium carbonate, Na_2CO_3, in about 400 mls. of distilled water in a beaker and pour into a liter volumetric flask. Add 7.5 gs. tartaric acid and dissolve. Add 4.5 gs. of $CuSO_4.5H_2O$, highest purity. When the copper salt has dissolved, make up to 1 liter with distilled water. After the solution has stood for a few days, decant the clear supernatant fluid from the whitish residue into another bottle.

(3) Special phosphomolybdic acid solution: Transfer to a liter beaker 35 gs. of molybdic acid, MoO_3. Dissolve in 200 mls. of distilled water and then add 5 gs. of sodium tungstate, $Na_2WO_4.2H_2O$. Add 200 mls. of 10 per cent. NaOH solution (page 913). Boil vigorously for half an hour in order to remove excess ammonia introduced in the molybdic acid. Cool. Transfer to a 500-ml. volumetric flask. Dilute to about 350 mls., and add 125 mls. of 85 per cent. syrupy phosphoric acid, H_3PO_4. Mix. Dilute to the mark.

Folin's Tyrosin Reagent.—See page 909, phenol reagent.

Folin's Bichromate Standard for Creatinin Determinations.—Half-normal potassium dichromate solution: Weigh, accurately, 24.55 gs. of highest purity $K_2Cr_2O_7$ and place in a liter volumetric flask. Dissolve in about 200 mls. of distilled water and make up to the mark with more water. This solution has approximately the same color as 10 mgs. of creatinin treated according to the Folin creatinin procedure. If the colorimeter standard cup is placed at 8.0 mms., and the calculation is made as if it were 8.1, quantitative relations hold.

Folin's Protid Standard for Quantitative Determination of Urinary Protid.—Dilute sheep-blood serum with 7 volumes of a solution of NaCl containing 15 gs. per 100 mls. of liquid. The solution is about 1 per cent.

Formalin.—40 grams dry H.CHO gas dissolved in water at 25° C.

Fuchsin-sulphurous Acid.—(See LaWall.) Weigh 0.5 g. basic fuchsin and add to 500 mls. water. Add 9 gs. sodium bisulphite ($NaHSO_3$), then 10 mls. conc. HCl, and finally 5 mls. sulphurous acid (SO_2, 6 per cent. in water; a commercial product). This is the U. S. P. "Fuchsin sulphurous acid T. S. (test solution)."

Fusion Mixture.—To 2 parts by weight of pure potassium nitrate, KNO_3, add 1 part sodium carbonate, anhydrous, Na_2CO_3. Mix well in a mortar and store away from fumes.

Gies' Biuret Reagent.—Weigh 3 gs. $CuSO_4.5H_2O$ crystals and dissolve in warm water (50 mls.). Make up to 100 mls. with cold water. Pipette 25 mls. of this copper solution into a 1000-ml. volumetric flask and add clear 10 per cent. KOH solution to the mark (1000 mls.).

Glucose for Tolerance Test.—100 gs. pure glucose dissolved in water and taken by mouth. For intravenous tolerance test on experimental animals inject 0.7 g. glucose in sterile aqueous solution per kilo body weight. Increase the concentration to 0.80 g. per kilo, which represents the average tolerance for the animal. Glucose is obtainable in pure form for intravenous work on the market, but in some samples there is unneutralized H_2SO_4 left from the method of hydrolysis; such preparations must not be used. For the use of glucose with insulin, see page 918.

Glycerol Solution.—Special for Leffmann-Beam method for Reichert-Meissl Number (see page 919).

Guaiaconic Acid Solution.—To 0.5 g. gum guaiacum add 100 mls. 95 per cent. ethanol.

Guenzberg's Reagent.—Dissolve 2 gs. phloroglucinol[1] and 1 g. vanillin[2] in 30 gs. of "absolute" ethanol. The solution does not keep well, especially in the light.

Heptylic (Cetyl) Alcohol, Antifoaming Mixture.—Add 25 gs. of spermaceti wax to 100 mls. of alcoholic sodium hydroxid (page 889). Boil over a water-bath or electric stove for one hour. Leave overnight. Then add 1 volume of distilled water, mix well, filter through paper and save the residue, cetyl alcohol. Transfer the waxy residue to a beaker, add 95 per cent. ethanol, dissolve, and let stand in a warm place; the alcohol, $C_{16}H_{33}OH$, crystallizes out. For use, make a thick solution in ethanol "absolute." The crystals melt at 50° C. They are easily removed by ethanol. Caprylic alcohol is on the market for similar purposes; it is liquid at ordinary temperatures and boils at 175° C.[3]

Hopkins-Cole "Glyoxalic" Reagent.—Method by reduction of oxalic acid: Into a tall cylinder place 500 mls. of saturated solution (1:5) of oxalic acid. Add 40 gs. of 2 per cent. sodium amalgam (page 924); hydrogen gas will be evolved. When the evolution of gas ceases, filter the solution and add 1 liter of distilled water. Keep well stoppered; a small amount of $CHCl_3$ may be added.

Method by magnesium reduction of oxalic acid: Place in an Erlenmeyer flask 10 gs. magnesium powder, used by photographers, and cover with distilled water. Add, slowly and with constant agitation of the contents of the flask, 250 mls. of saturated oxalic acid. Cool under the tap while the additions are being made. After the evolution of H_2 has ceased, filter, acidify with a few drops of glacial acetic acid, and dilute to 1000 mls. with distilled water. Preserve as directed above.

Method by electrolysis: Arrange two platinum electrodes protected from the house-current by a series of lamps or other resistance, so that they may dip in a saturated solution of oxalic acid. Pass the current through the solution until there is no longer a conspicuous evolution of gas from the solution. A yield of about 87 per cent. is obtained in this way. Preserve as directed before.

Hopkins-Cole Reagent Used in Tyrosin Determination.—Page 271; $HgSO_4$, 1000 gs.; H_2O, 500 mls. Add 70 mls. H_2SO_4 and dilute to 1000 mls. with distilled water.

Hopkin's Lactic Acid Reagent.—Thiophen.

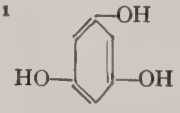
Hydrochloric Acid, Concentrated, Colorless.—36 per cent. HCl, water 64 per cent. Specific gravity, 1.18. Free chlorin, 0.0002 per cent. Chief impurity, SO_2.

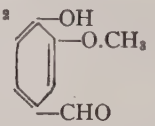
Hydrochloric Acid, Dilute.—10 per cent. solution. Specific gravity, 1.05.

Hydrochloric Acid, Normal Solution.—Dilute 105 mls. concentrated hydrochloric acid up to 1000 mls. with distilled water; this gives an approximately normal solution, slightly stronger than the theoretical. The normality must be determined by titrating against standard alkali. Percentage dissociation, 0.1 normal solution at 25° C., 91 per cent.

Hydrogen Sulphide for the Laboratory.—In the middle compartment of a Kipp generator (Fig. 263, page 893) place a number of pieces of ferrous sulphide sticks or lumps. Into the upper compartment pour dilute sulphuric acid until the liquid rises against the sticks of FeS in the middle compartment. Reaction: $FeS + H_2SO_4 = H_2S\uparrow + FeSO_4$. The gas leaves the side-tube when the cock is opened.

Individual method by asbestos preparation of "Aitchtuess."[4] Place a cylinder about 2 cms. deep of the material in the bottom of a Pyrex test-tube. Fit a glass

1 

2 

[3] This is the Eastman Kodak Co., Rochester, N. Y., preparation; see page 295.

[4] Obtainable from the Philadelphia Special Chemical Co., 1833 Chestnut Street, Philadelphia, Pa.

delivery tube ("L"-shaped) to the mouth of the test-tube by means of a rubber stopper. Fix the test-tube horizontally upon a ring stand by means of a burette clamp and apply a low flame of a microburner (Fig. 248, page 841) to the end of the test-tube containing the asbestos preparation. Collect the gas as desired, but remove the flame when the gas is no longer to be used. *Do not permit the gas to go out into the room.* Remember that as the tube cools back suction will take place.

Hypobromite Solution for Urea Determination.—Sodium hypobromite: Dissolve 100 gs. of NaOH in 250 mls. of distilled water. Cool. Add, with care, 25 mls. of liquid bromin. Cool thoroughly under the tap. The solution does not keep and must be freshly prepared when desired for use.

Indicators for pH Determinations:

Clark-Lubs Indicators.—Method for preparing indicator solutions from the dry powders:

Phenol red: Grind, in a mortar, the contents of a bottle (10 decigrams) with 5.7 mls. twentieth normal sodium hydroxid solution. Dilute to 25 mls. with distilled water.

Bromphenol blue: Repeat as for phenol red, but use 3.0 mls. of the alkali.

Cresol red: Repeat, using 5.3 mls. alkali.

Bromcresol purple: Repeat, using 3.7 mls. alkali.

Thymol blue: Repeat, using 4.3 mls. alkali.

Bromthymol blue: Repeat, using 3.2 mls. alkali.

Methyl red: Repeat, using 7.4 mls. alkali.

Lamotte Standard Indicators.—Mix 0.5 ml. of the prepared commercial solution of the following indicators, with 10 mls. of the special buffer solutions, M/5:

Thymol blue (acid range).

Bromphenol blue.

Bromcresol green.

Bromcresol purple.

Bromthymol blue.

Phenol red.

Cresol red.

Thymol blue (alkaline range).

Insulin is prepared for the market by several pharmaceutical firms, being concessionaries of the University of Toronto, which holds the patent. While the methods of preparation differ and are constantly being modified, the general procedure is as follows: Beef pancreas is ground, treated with sulphuric acid, then with ethanol and centrifuged, the supernatant fluid removed and saved as the "first extract." To the residue are added H_2SO_4 and ethanol and centrifuged again, the supernatant fluid being united with the first extract, the sediment being discarded. The extracts are filtered, and NaOH added. The mixture is subjected to vacuum concentration at below 30° C. Ammonium sulphate is added to precipitate the extract. The precipitate is collected and dissolved in dilute NH_4OH. Hydrochloric acid is added and then ethanol to precipitate the protids. Ether is added to the filtrate and after an exposure to low temperature for some time, there settles out a jelly which is dissolved in acid and the reaction adjusted to the iso-electric point (5.0–4.7). The product is grayish. The concentrated product is brought to *p*H 2–2.5 and isotonic with physiologically normal saline (0.9 per cent. NaCl) solution. It is sterilized by means of a Berkefeld filter and subjected to standardization tests. For the market it is prepared in ampoules of "U-10" containing 10 units[1] to the ml.; "U-20," containing 20 units to the ml.; "U-30," 30 units to the ml., and "U-40," with 40 units to the ml. The unit is defined as that dose administered subcutaneously to a 2-kilogram rabbit, having been fasted for twelve hours, which will induce a fall of

[1] Clinical units.

blood-sugar to 0.045 g. per 100 mls. of whole blood within four hours and result in hypoglycemic convulsions (Fig. 160). The formula used is:

$$\text{Units of insulin per ml.} = \frac{\text{Reduction of b. s.}}{\text{Diff. bt. normal and convulsive blood-sugar level}} \times \frac{\text{kilos body wt.}}{\text{mls. insulin used}} \times 1.5.$$

The insulin is finally tested for precipitation by subjecting it to high temperatures for two days. If no precipitation occurs, the sample is released for sale. The standardization varies from 5 to 10 per cent., so that each lot varies to a small extent and must be used accordingly. The blood-sugar figures serve as faithful indices of the condition of the patient. If there is a tendency to hypoglycemia after administrating insulin, glucose must be given either by mouth or 5 to 20 gs. must be given by injection by vein. Insulin is given a half-hour before meals once or more times daily. Insulin is a dangerous substance and must be administered only at the advice of a physician.

Iodid, Potassium, by Mouth for Motility Test.—0.3 g. in capsules.

Iodin Solution, Potassium Iodid.—Decinormal solution: Dissolve 13 gs. iodin in 30 gs. KI dissolved in 30 mls. of distilled water. Make up to 1 liter. The solution is then standardized against decinormal thiosulphate (page 926).

Iodin (Lugol's Solution).—Five per cent. iodin in a 10 per cent. KI solution (aqueous).

Iodin, Tincture.—Seven per cent. iodin in 5 per cent. alcoholic KI solution.

Iodin Solution for Aceton Determinations.—Weigh 10 gs. KI into a beaker and add 50 mls. of distilled water. Weigh accurately 6.4 gs. iodin into a beaker and cover with a watch-glass while weighing. Add this to the KI solution. Dissolve and then transfer the solution to a 500-ml. flask, dilute to the mark, and mix. Standardize against thiosulphate solution.

Kraus' Tryptophan Method (page 314).—*Reagents:* Toluene pure, *i. e.* toluene that gives no color with the vanillin-HCl reagent. HCl concentrated (specific gravity 1.19). H_2SO_4 50 per cent. by volume. H_2SO_4 5 per cent. by volume. Vanillin 0.5 in 50 per cent. acetic acid. Mercuric sulphate reagent—10 per cent. HgSO, made by triturating 10 grams $HgSO_4$ with portions of 5 per cent. H_2SO_4 until dissolved and made up to 100 mls. Mercuric sulphate—2 per cent.—made as above by dissolving 2 grams $HgSO_4$ in 100 mls. of 5 per cent. H_2SO_4.

Lange's Colloidal Gold Reagent.—Vessels must be absolutely clean; wash glassware with aqua-regia (page 890), then with tap, and then with distilled H_2O. Distill a liter of ordinary distilled water through Pyrex or quartz vessels. Heat 500 mls. to 60° C. Add 5 mls. of 1 per cent. "gold chlorid";[1] then 5 mls. of 2 per cent. K_2CO_3. Bring the temperature to 90° C., but no higher, and then withdraw the flame. Add 5 mls. of a 1 per cent. solution of best grade commercial "formalin," made by diluting 1 ml. of the formalin from a freshly opened bottle up to 100 mls. double distilled water. Agitate the solution of gold while adding the formalin until the solution begins to turn pinkish, after which more formalin in larger amounts are added until a deep red color appears. There may be no purple in the color. Test: 1.7 mls. of 1 per cent. NaCl ppt. 5 mls. in sixty minutes.

LaWall's Method for Methanol in the Presence of Ethanol.—See page 919: Dilute the alcohol solution to contain about 5 per cent. ethanol by volume. To 5 mls. of this diluted solution, in a 25-ml. volumetric cylinder, add 5 drops phosphoric acid solution and 2 mls. of a 3 per cent. aqueous solution of potassium permanganate. Let

[1] Commercial gold chlorid solutions are composed of $AuCl_3.HCl.3H_2O$, containing about 50 per cent. Au. The crystals are bright yellow. They are usually supplied in ampoules containing 0.9 gram. To make a 1 per cent. solution dissolve the contents of an ampoule in about 50 mls. of double distilled water and make up to 90 mls. with the water.

stand for ten minutes. Add 1 ml. 10 per cent. aqueous solution oxalic acid. Let stand until the solution takes on a transparent brownish color. Now add 5 mls. diluted and cooled sulphuric acid (1:3) solution in water and finally 5 mls. of the special fuchsin-sulphurous acid solution (see page 915). Mix and let stand for ten minutes. When viewed against a white back-ground, methyl alcohol imparts a violet color. A control should be conducted: Repeat the procedure, but add 2 drops of a 5 per cent. methanol solution. Sensitivity, 1:10,000.

Lead Acetate, Normal Solution.—190 gs. $Pb(C_2H_3O_2)_2 3H_2O$ dissolved in water and made up to 1000 mls.

Lead Acetate ("Basic").—0.5 normal: Dissolve 56 gs. PbO (yellow lead oxid or "litharge") in about 200 mls. of distilled water and add 95 gs. of lead acetate, $Pb(C_2H_3O_2)_2.3H_2O$; make up to 1000 mls. with distilled water.

Lead, powdered, for creatin determinations, see page 890.

Leffmann and Beam's Special Glycerol Reagent for Reichert-Meissl Number.—Add to 180 mls. of pure glycerol 20 mls. of 50 per cent. NaOH solution.

Liebermann's (Burchard) Reagent.—Acetic anhydrid, specific gravity 1.08; chloroform, free from chlorin.

Lime-water.—Dissolve an excess of calcium oxid (unslaked lime) in water, shake well, and leave well stoppered for several days; then decant the pure supernatant liquid from the residue. Preserve in a vessel so that CO_2 cannot contaminate the solution.

Lloyd's Reagent.—Obtainable from J. U. Lloyd Co., Cincinnati, Ohio.

Mastic Suspension for Colloidal Demonstrations.—Dissolve 5 mastic "tears" in "absolute" ethanol and add the solution to 50 mls. of distilled water; a milky suspension occurs.

Magnesia Mixture.—Dissolve in distilled water (200 mls.) 55 gs. of magnesium chlorid, $MgCl_2.6H_2O$, and after solution, 70 gs. ammonium chlorid (NH_4Cl). Then add 125 mls. of ammonium hydroxid, concentrated, specific gravity, 0.880. Make up to 1000 mls. with distilled water.

Mayer's Reagent.—Dissolve in 200 mls. of distilled water 13.55 gs. mercuric chlorid, $HgCl_2$, warming the solution if necessary. Then add 49.80 gs. of potassium iodid, KI, and then water to make a liter of solution.

Mercuric Chlorid Normal Solution.—Weigh 136 gs. $HgCl_2$ and dissolve in 800 mls. of distilled water. Make up to 1000 mls. with water.

Methanol in the Presence of Ethanol.[1]—Place in a 200 by 20 Pyrex test-tube free from scratches 1 ml. of the suspected alcohol, 2 mls. of 6.7 per cent. potassium dichromate solution, and 2 mls. of 1 to 2 (62 per cent.) sulphuric acid. Mix. Allow to stand at room temperature for about ten minutes. The reduction of the chromic acid to blue chromic sulphate should take about forty seconds, and if it takes much longer than this the alcohol used contains too much water. Add 15 mls. of distilled water, mix thoroughly, and heat in a boiling water-bath for ten minutes. Now add 5 mgs. of orcinol in 1 ml. of water, mix very thoroughly, and heat in the boiling water-bath for thirty minutes. If the alcohol contained 5 per cent. or more of methanol, a precipitate will be formed after about five minutes' heating. With 1 per cent. of methanol a precipitate will form after fifteen minutes' heating. The test will show methanol down to 0.5 per cent., although in this case it may be necessary to heat for thirty minutes and then allow the solution to cool before a precipitate forms. If the precipitate is filtered off it will be seen to be distinctly brown or yellow.

Quantities of methanol smaller than 0.5 per cent. can be detected by precipitating the chromium by adding a slight excess of sodium hydroxid and heating. When this is filtered, the clear filtrate possesses a green fluorescence if even traces of methanol were originally present. This last procedure is of doubtful value, as it

[1] J. B. Sumner, Jour. Amer. Chem. Soc., vol. 45, p. 2378, 1923.

is to be expected that traces of methanol derived from glucids like pectins may possibly be present in beverages from fruits.

The alcohol used in the test is obtained by distilling the suspected solution or beverage, using a glass column to obtain as complete a separation from the water as possible. The temperature of the upper portion of the column should not be allowed to exceed 80° C.

Formic acid, amyl alcohol, acetone, and furfural do not interfere with the test. Glycerol does not interfere because it is eliminated in the process of distillation; but if it is added to the alcoholic distillate it gives a positive test if as much as 5 mgs. are present.

Mette Tubes for Digestion Experiments.—By means of the back of a pocket knife or similar object break a fresh egg in its equator. Slip the tips of your thumbs into the incision and carefully pry the two halves apart, catching the yolk in one-half and permitting the egg-white to drain into a jar. Repeat, using 3 eggs in all. Save the yolks for experiments on lecithin; the instructor will designate a place for them, preserved by alcohol. Then, by means of a bunch of wires or an egg-beater, break up the egg-white; strain through glass wool and use the more liquid portion passing through the funnel for the making of the tubes: Secure glass tubing about 1.5 mm. inner diameter and with heavy walls. Cut into decimeter lengths. Suck into each one egg-white until they are filled. Lay the tubes horizontally upon a piece of gauze, wrap them, and tie the gauze into a bag. Lower this bundle into your saucepan or casserole in which water has been boiled and permitted to cool to 85° C. Leave until the water has cooled to room temperature (20° C.). Remove the tubes from the water, unwrap the gauze, and dry the exterior of the glass-tubes on filter-paper. Melt some 60° C. paraffin and dip the extreme tip of the tubes into it, in order to protect the tubes from drying. They maybe stored in this way in a refrigerator at about 8° C. When ready for use, cut about 1 cm. from each end, discard, and use the more central portions of the tubes for enzyme work.

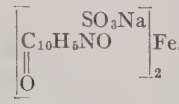
Millon's Reagent.—Dissove 1 part by weight of mercury in 2 parts by weight of pure white nitric acid, HNO_3, specific gravity 1.42. A hood should be used. After the reaction has progressed for some time, cautiously heat the vessel containing the solution and dilute with twice its volume of distilled water. Leave overnight, filter, and store. This is a solution of HNO_3, HNO_2, $Hg_2(NO_3)_2$, and $Hg(NO_3)_2$.

Moerner's Reagent.—Mix 1 ml. of commercial formalin with 45 mls. of distilled H_2O and add, cautiously, 55 mls. of 50 per cent. H_2SO_4 solution.

Molisch Reagent.—See α-naphthol solution, page 889.

Molybdate, Alkaline.—See page 891.

Myers' Naphthol-green Standard for Cholesterol Determinations.—Weigh 50 mgs. of the dye Naphthol Green B and dissolve in water to make 1000 mls. solution. Standardize against 5 mls. of a chloroform solution of cholesterol containing 0.4 mg. cholesterol. Naphthol Green B is:



Myers' Permanganate Solution for Calcium Determinations.—Fill to the mark a 100-ml. volumetric flask with 0.1 normal $KMnO_4$ and dilute to 1000 mls. in a liter flask.

Nitric Acid, Concentrated.—Specific gravity, 1.42, 65 per cent. HNO_3. Clear, colorless before opening container, but turning brownish to reddish yellow on exposure to air, due to the formation of oxids of nitrogen. Chief impurity: calcium and N_2O_4.

Nitric Acid, Dilute.—10 per cent. solution from concentrated. Specific gravity 1.06.

Nitric Acid, Normal Solution.—250 mls. of concentrated nitric acid made up to 1000 mls. with distilled water. Percentage dissociation at 25° C. 0.1 normal, 92 per cent.

Nitric Acid, Fuming.—Specific gravity, 1.50. Yellowish to brownish red. Contains N_2O_4, NO_2, etc. Fuming nitric acid can be made for immediate use by boiling concentrated nitric acid with a match-stick or piece of pine wood.

Nylander's Solution.—Dissolve 50 gs. Rochelle salt (potassium sodium tartrate) in 500 mls. of distilled water and add 20 gs. of bismuth subnitrate or subcarbonate, basic nitrate, $(Bi(OH_2)_2NO_3)$; basic carbonate, $(2(BiO)_2CO_3.H_2O)$. Add 80 gs. NaOH purified by alcohol, and make up to 1000 mls. when cooled to room temperature.

Obermayer's Reagent.—Dissolve 4 gs. ferric chlorid, Fe_2Cl_6, in 400 mls. of concentrated hydrochloric acid and make up to 1 liter with the acid.

Pavy's Solution.—Mix 120 mls. of complete Fehling's solution (page 907) with 300 mls. concentrated ammonium hydroxid, specific gravity, 0.880. Make up to 1000 mls. with distilled water.

Pepsin Solution.—Method of preparation from gastric mucosa: Secure from the abbatoir pigs' stomachs and leave them for an hour or so in a warm place for autolysis to take place to a certain extent. Then open the stomachs, make long cuts through the mucosa, and tear it from the muscular coats. Pass the strips through a meat-grinder and add the mash to 300 mls. of 0.1 normal HCl solution in a large salt-mouth bottle. Leave in a cool place overnight, insuring that the mucosa comes into intimate mixture with the acid solution. Strain off the mucosa through gauze and save the liquid as an HCl extract containing pepsin. Place the mucosa in glycerol and leave indefinitely; the glycerol extract, when used for pepsin, must have a 0.2 per cent. HCl solution added.

Pepton, Witte's.—To show pepton shock in mammals: Inject per kilo of body weight 0.2 to 0.5 g. dry pepton or 2 to 5 mls. of 10 per cent. solution in water.

Permanganate, potassium, for calcium determinations, see page 920.

For decinormal solution: Weigh 3.161 gs. pure, preferably long crystal $KMnO_4$ (better expressed as $K_2Mn_2O_8$, since it is in this form that the permanganate participates in most reactions) and make up to 1000 mls. H_2O. Calculation (in acid solution): $K_2Mn_2O_8 + 4H_2SO_4 + 2KHSO_4 + 2MnSO_4 + 3H_2O + 5O$; hence $316.06 \times 0.5 \times 0.2 \times 0.1 = 3.16$ gs: liter.

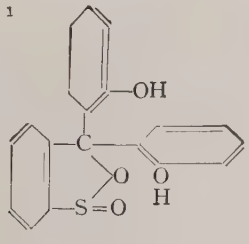
For antidote for poisons: By stomach-tube per kilo, 15 mls. of 1 per cent. permanganate aqueous solution.

For alkaline permanganate solution in gas analysis: 50 gs. of $KMnO_4$ crystals are dissolved in 700 mls. of warm (60° C.) distilled water. Cool. Weigh 25 gs. of KOH and dissolve in the permanganate solution. Make up to a liter with more water.

Potassium permanganate is soluble in water to the extent of 7 gs. per 100 mls., at room temperature, 20° C.

Phenol Reagent.—See pages 892 and 909.

Phenolsulphonephthalein[1] *for Renal Efficiency Test.*—The usual commercial ampoule contains more than 1 ml., but 1 ml. contains 6 mgs. of the dye as mono-sodium-phenol-sulphone-phthalein. For injection in renal efficiency test: By means of a



small file break the tip of the ampoule at the constriction. Insert a sterile hypodermic needle attached to a syringe and withdraw exactly 1 ml. Injection is made into the lumbar or deltoid muscles. The test is performed by making colorimetric comparison of the urine taken at one-, two-, and three-hour intervals, each specimen being made up to 1000 mls., against the standard. Standard: Dilute exactly 1 ml. of the dye up to 1 liter with distilled water.

Phenolphthalein, Indicator.—Weigh 1 g. and dissolve in ethanol, 50 per cent., to make 100 mls.

Phenolphthalin (Not Phenolphthalein).—Blood test: See pages 396 and 397.

Phlorhizin[1] diabetes in dog, by vein, per kilo, 0.1 g. For rabbit, 0.25 g ; it is a glucoside, glucose being united to phloretin (ester of mono-phloretic phloroglucinol). It is obtained from cherry bark. See page 195.

Phosphates, Solutions Required for Analysis.—(1) Acetate solution: Dissolve 100 gs. of sodium acetate in distilled water and add 100 mls. of strong (glacial) acetic acid. Make up to 1000 mls. (2) Standard potassium phosphate solution: Pipette 28.17 mls. of 0 2 molecular KH_2PO_4 into a 100-ml. volumetric flask and make up to the mark with distilled water. (3) Standard uranium acetate solution: To about 500 mls. of distilled water add 36 gs. of uranyl acetate,[2] applying heat if necessary. Cool, make up to 1 liter, and filter. (Page 901.)

Phosphates, Special Reducing Reagent of Fiske and Subbarow.—(See page 792; amino-naphthol-sulphonic acid.) This reagent may be prepared as described for amino-acids (p. 911) in which case it should be washed with ethanol until no more color is removable; or, it may be made by recrystallizing the Eastman Kodak Co. No. T360: Pour a liter of dist. H_2O into a 1.5 liter, or larger, Erlenmeyer flask. Heat just below boiling (90° C.). Dissolve in the water 150 gs. sodium bisulphite (see page 924) and then 5 gs. of dry sodium sulphite (freshly opened). Next add 15 gs. of the technical amino-naphthol-sulphonic acid. Agitate until all ingredients are dissolved. Filter while hot in a large funnel. Cool the filtrate and add 10 mls. conc. HCl. Using a Buchner funnel (Fig. 261), filter off the precipitate and wash with 300 mls. H_2O and then with 95 per cent. ethanol until the filtrate is colorless. Dry the residue and store in an amber bottle.

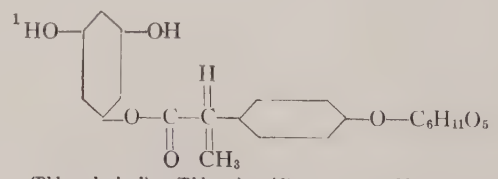
Solutions for buffers; see page 901.

Phosphorus for Inducing Hepatic Degeneration.—Administer by stomach 10 gs. white or yellow phosphorus per kilo, wrapped in food or in albolene or other oil, by stomach-tube.

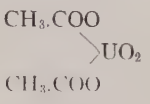
Picric Acid.—Saturated at 20° C., 2 per cent. solution. Purification, see page 891.

Potassium Chlorid, Normal Solution.—Dissolve 74.5 gs. KCl in distilled water and make up to 1 liter.

Potassium Dichromate, $K_2Cr_2O_7$, Normal Solution.—Weigh 49.10 gs. and make up to 1 liter. $K_2Cr_2O_7$ gives up 3O = 6H; therefore take $\frac{1}{6}$ mol. wgt. Fundamental equation: $K_2Cr_2O_7 + 6FeO = 3Fe_2O_3 + K_2O + Cr_2O_3$. See page 736.



(Phloroglucinol). (Phloretic acid). (Glucose).



Potassium Ferrocyanid, $K_4Fe(CN)_6.3H_2O$, *Normal Solution.*—Dissolve 106 gs. in enough distilled water to make 1000 mls.

Potassium Ferricyanid, $K_3Fe(CN)_6$ *or* $K_6Fe_2(CN)_{12}$, *Normal Solution.*—Weigh 22 gs. and dissolve in enough water to make 1000 mls. solution.

Saturated solution: 43 gs. ferricyanid up to 100 mls. H_2O at 25° C.

Potassium Oxalate, $(COOK)_2.H_2O$, *Normal Solution.*—Weigh 83.01 gs. and make up to a liter with distilled water.

As anticoagulant 1 g. made up to 100 mls. distilled H_2O. See page 71.

Potassium Permanganate.—See page 921.

Potassium Acid Phthalate.—For chemical formula see page 897. Properties: Non-hygroscopic, water soluble, behaving as a monobasic acid, colorless. Solubility at 25° C., 10.24 per cent.; 35° C., 12.67 per cent.; 100° C., 36.12 per cent. Solutions should be made in water not above 35° C., as supercooling results, producing a more acid salt. References: Journal Industrial and Engineering Chemistry, vol. 7, page 29, 1915; Jour. Amer. Chem. Soc., vol. 37, page 2352 (Hendrixon), 1915; vol. 42, page 724, 1655 (Hendrixon and Dodge, respectively), 1920.

Use phenolphthalein for titrations with potassium acid phthalate. Sodium alizarin sulphonate cannot be used.

Potassium Thiocyanate, KCNS, Decinormal Solution.—Dissolve 10 gs. of the crystals in water sufficient to make 1 liter of solution. This solution is approximately decinormal; for exact normal it is necessary to standardize it (page 790). Decinormal $AgNO_3$ solution = 16.989 gs. $AgNO_3$ per liter of solution. For Volhard and modifications of this method for chlorids, see pages 788, 790, 924, and 931.[1]

Quinhydron, Preparation from hydroquinon.—Weigh 160 gs. hydroquinon and dissolve in 150 mls. of distilled water at 60° C. Warm, in another flask, 300 mls. of distilled water and dissolve in it at 60° C., 100 gs. iron-alum (ferric-ammonium sulphate, violet crystals). Filter. Pour the alum solution into that of the hydroquinon. Mix and let stand until a maximum crop of quinhydron crystals separate. Place the flask in chipped ice for a few minutes, not permitting the water to freeze; then pour the mixture of crystals upon a Buchner funnel and suck dry. Wash three times with ice-water. Let the crystals dry in the air and continue drying in a desiccator.

Robert's Reagent.—Add 1 volume pure white nitric acid to 5 volumes of a 40 per cent. (saturated) solution of magnesium sulphate.

Saline, Sodium Chlorid Physiologically Normal.—For frog, 0.5 per cent. NaCl; for mammal, 0.9 per cent. NaCl. Weigh 6 (or 9) gs. NaCl and make up to 1000 mls. Special, for blood dialysis: Eight gs. NaCl made up to 1000 mls. with distilled water. Test for free acids: Place 5 mls. of the well-mixed solution in a Pyrex test-tube and add 2 drops of phenol red indicator; the solution turns yellowish. Boil, expelling the CO_2; the color changes to an orange red if the solution is free from acids other than CO_2. If the solution appears pinkish, an alkaline reaction is present and the solution must be adjusted.

Santonin, for demonstrating color of the urine after using this vermifuge: Man, by mouth, 0.05 g.; cat, hypodermic per kilo, 500 mgs. Rabbit, per kilo, stomach-tube, 2.0 gs. Fatal for man, 200 mgs.

Schiff's Reaction for Aldehydes.—Reagent: Acid fuchsin, 1 per cent. aqueous; bubble SO_2 gas through the solution until it has become decolorized. For SO_2 see page 926. For other aldehyde reactions see pages 893 (Bial) and 941.

Schweitzer's Reagent.—Add about 5 gs. of ammonium chlorid to 100 mls. of a 5 per cent. cupric sulphate solution and add sodium hydroxid, 10 per cent.; a blue precipitate is formed. Filter, saving the residue. Wash on the paper with water, press through several layers of filter-paper, and dissolve in ammonium hydroxid solution, concentrated.

[1] M. L. D. (minimal lethal dose), 15 gs. in man.

Scott-Wilson Reagent.—See page 888.

Seelman Reagents for Modified Volhard Method for Chlorids.—(1) Weigh 29.055 gs. silver nitrate and dissolve in about 900 mls. of 25 per cent. HNO_3 solution. Add 50 mls. of saturated[1] ferric-ammonium sulphate, $FeNH_4(SO_4)_2.12H_2O$, violet crystals. Make up to 1000 mls. with water. (2) Weigh, roughly, 8 gs. of ammonium thiocyanate, $NH_4.CNS$, and make up to 1000 mls. Make 2 mls. of Solution 2 = 1 ml. of Solution 1, by following the method of titration on page 788.

Selivanoff's Solution.—Dissolve 0.05 g. resorcinol[2] in 200 mls. of 50 per cent. hydrochloric acid.

Silver Nitrate Solution, Decinormal.—Weigh 16.98 gs. of crystals[3] and dissolve in enough distilled water to make 1000 mls. solution.

For the Volhard-Harvey method weigh 23.94 gs. silver nitrate crystals and make up to 1000 mls. with distilled water. Each ml. = 8.23 mgs. of NaCl.

Silver Nitrate Solution, Ammoniacal.—Ammonium hydroxid is added to a 10 per cent. solution of $AgNO_3$, making $Ag(NH_3)_2NO_3$.

Sodium Amalgam.—Weigh, quickly, 15 gs. of metal sodium cut by means of scissors from a stick of sodium under paraffin oil. Add, slowly, to 1000 gs. of mercury. The temperature will rise to about 150° C. After the amalgam has formed and cooled, cover it with distilled water and knead it with a glass rod under the water. Then pour off the water and dry the amalgam between several layers of filter-paper. For pure sodium amalgams see Richards and Conant, Jour. Amer. Chem. Soc., vol. 44, page 602, 1922.

Sodium bisulphite is being replaced on the market by the pyrosulphite or meta-bisulphite, for keeping qualities. Either may be used, interchangeably.

Sodium Chlorid, Saturated Solution.—35.6 gs. of NaCl per 100 mls. of distilled water at 25° C.

Normal solution, 58.50 gs. NaCl made up to 1000 mls. H_2O. Specific gravity 1.378 at 20° C.

Physiologically normal NaCl solution, see page 923.

Sodium Cobalti-nitrite, $Na_3Co(NO_2)_6$.—It may be made as follows: Dissolve 30 gs. sodium nitrite, $NaNO_2$ in about 50 mls. distilled water. Add 5 gs. cobalt nitrate and 2.5 mls. glacial acetic acid. Make up to 100 mls. with distilled water. Filter after one day from any residue.

Sodium Nitrite Solution, $NaNO_2$.—For general purposes decinormal solution, 6.9 gs. per 1000 mls. of solution.

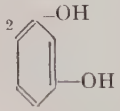
For the Van Slyke gasometric method: Make 30 gs. of $NaNO_2$ sticks up to 100 mls. of distilled water.

Sodium Phosphate Solutions for Buffers.—See page 901.

For normal di-sodium-monohydrogen phosphate, $Na_2HPO_4.12H_2O$, weigh 119.5 gs. of the crystals and make up to 1000 mls. with distilled water.

Sodium Ethylate Solution.—Dissolve about 2 gs. of sodium metal, cut by means of scissors from a piece of sodium metal under oil, in "absolute" ethanol, and after dissolution of the sodium add enough "absolute" ethanol to make 1000 mls. solution. Standardize against a decinormal solution of benzoic acid in chloroform. One ml. of decinormal sodium ethylate = 1.79 mgs. of hippuric acid (page 741).

[1] 35 gs. of crystals made up to 100 mls. H_2O at 20° C.



[3] Or 17.1 of fused $AgNO_3$, since on fusion in air some of the $AgNO_3$ becomes silver oxid, Ag_2O, which, in the presence of HNO_3, does not form silver chlorid.

Soluble Starch, Lintner's Method; Modified by Small.—Potato starch. Older potatoes are scrubbed with a vegetable brush and passed through a meat-grinder with the appropriate grinder. Transfer the grindings to a large piece of scrim, and by manipulation work out the starch from the fibers by submerging the bag under the surface of distilled water in a large vessel like an evaporating dish. Let the fluid in the vessel remain for an hour or longer in order that sedimentation of the starch and fibers that accompany the starch through the mesh may afford a means of separating the two layers. Decant the supernatant liquid from the fibers; the decanted fluid contains the starch. Filter the starch from the fluid, discarding the filtrate, and leaving the starch on the paper exposed to air until dry. Weigh 25 gs. of the starch into a liter flask. Add 40 mls. of distilled water and 15 mls. of concentrated hydrochloric acid. Agitate the fluid to insure complete mixture. Leave one week, then pour off the supernatant fluid, and wash the residue with several changes of water by decantation. Filter through a Buchner funnel with hardened filter-paper. Suspend the residue in a buffer solution the reaction of which is *p*H 7.0 (see page 901). Before use, decant the supernatant buffer solution from the starch, add 1 volume distilled water, and filter on a Buchner funnel with hardened paper. Dry in the air. Or: The above procedure may be followed as far as the addition of distilled water and leaving one week, replacing this procedure by the following: Weigh 100 gs. of the starch that has been air dried. Add 500 mls. 95 per cent. ethanol to which 4 mls. of concentrated hydrochloric acid have been added and place the vessel holding the solution in a boiling water-bath for fifteen minutes, agitating the contents at intervals. A reflux condenser of the Hopkins type should be used. Filter, wash with several changes of distilled water, and dry in the air.

Sørensen Standard Solutions.—See page 899.

Spiegler's Reagent.—Mix in about 500 mls. distilled water:

Mercuric chlorid	40 gs.
Tartaric acid	20 gs.

When dissolved, add 100 gs. glycerol, then 50 gs. sodium chlorid.

Finally make up to 1000 mls. with distilled water.

Sodium Chlorid.—Special Solution for pH Determinations: "Physiologically Normal."—Isotonic solution used on page 923: Weigh 9 grams of pure crystal NaCl and dilute with distilled water to 1 liter. Add 2 drops of an 0.01 per cent. solution of phenol red.

Stokes' Solution.—Dissolve 3 gs. of ferrous sulphate, $FeSO_4.7H_2O$, in 50 mls. distilled water. Make a cold solution of tartaric acid, 2 gs. in 25 mls. distilled water. Add this to the ferrous sulphate solution. Mix. Make the solution up to 1000 mls. with distilled water. For use: Take 10 mls. of this solution and add concentrated ammonium hydroxid until the precipitate which forms when ammonia is added is dissolved. This must be done immediately preceding use.

Alternate method: Weigh 2 gs. of ferrous ammonium sulphate, $FeSO_4(NH_4)$-$SO_4.6H_2O$, into a 100-ml. volumetric flask and add about 50 mls. of distilled water. After dissolution add 3 gs. of tartaric acid. After complete solution add concentrated NH_4OH to clear the solution and water to make 100 mls. The solution keeps only a short time.

Sumner's Methanol Method.—See page 919.

Sumner's Reagents for Glucose Determination.—Dinitrosalicylic acid solution: Weigh ten grams of Eastman Kodak Co. No. 1802 or, 3–5, di-nitro-salicylic acid crystals and transfer to a liter flask. Dissolve by the aid of agitating the crystals with 500 mls. warm water. Make to the mark. Weigh 13.5 gs. stick NaOH rapidly on the laboratory scales and make up to 300 mls. with cold water in a 2 liter beaker. Add 880 mls. of the salicylic acid solution and mix. Then add 225 gs. potassium sodium tartrate ("Rochelle Salts") which has been finely pulverized in a mortar. Dissolve

by stirring and transfer to a bottle which can be tightly stoppered. Standard iron-alum solution: Weigh 345 mgs. of violet ferric ammonium sulphate crystals and transfer to a liter flask. Add 1 g. 3-5-di-nitrosalicylic acid and then 500 mls. water. Shake the flask until the solution is complete. The color of this solution is equivalent to a glucose solution containing 1 mg. treated by the Sumner quantitative method for sugar (page 778).

Sulphur Dioxid for Laboratory Purposes.—Place 50 gs. of freshly opened sodium bisulphite, $NaHSO_3$, in the middle compartment of a Kipp generator (page 893), and add through the opening into the upper compartment 500 mls. of 1:10 hydrochloric acid solution. Glass-wool packed around the stem of the tube passing from the upper compartment through the middle one prevents the bisulphite from passing into the lowest compartment. The equation is as follows:

$$NaHSO_3 + HCl = NaCl + H_2SO_3.$$

The sulphurous acid decomposes into H_2O and SO_2. The SO_2 gas escapes from the side tube of the middle compartment when the stop-cock is opened.

Sulphur dioxid is used to decolorize basic fuchsin in Schiff's test (page 923).

Starch, Soluble.—See page 925.

Sulphosalicylic Acid Solution.—Dissolve 13 gs. of salicylic acid in 20 mls. of concentrated sulphuric acid. Warm in order to effect solution and, after cooling, add 67 mls. of distilled water.

Sulphuric Acid, Concentrated.—Colorless, syrupy, specific gravity, 1.84. Ninety-four per cent. H_2SO_4. Chief impurity, lead.

Dilute: Ten per cent. aqueous, specific gravity, 1.069.

Normal solution: Dilute 28 mls. of concentrated sulphuric acid to 1000 mls. distilled water at 20° C. Per cent. dissociation at 25° C., decinormal solution, 58 per cent. Standardize against known alkali.

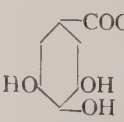
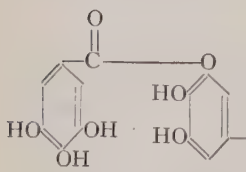
Fuming sulphuric acid: 15 per cent. SO_3.

Tannic Acid,[1] *Plimmer's Solution.*—Add to 500 mls. of distilled water 100 gs. of tannic acid and, after dissolution, 25 gs. of sodium acetate, then 25 gs. of sodium chlorid, and finally 50 mls. glacial acetic acid. Make up to 1 liter with distilled water.

Thiocyanate Solution for Volhard and Modifications.—For Volhard-Harvey method: Dissolve 8 gs. of ammonium thiocyanate, NH_4CNS, in about 800 mls. of distilled water and make up to 1000 mls. with distilled water. Titrate against standard silver nitrate solution (page 924) with a known NaCl solution as basis for standardization; for the method, see pages 788–790.

Seelman's modification: See page 788.

Thiosulphate Solution for Aceton Determinations.—Weigh 24.85 gs. of pure sodium thiosulphate, $Na_2S_2O_3.5H_2O$, from a freshly opened bottle. Make up to 500 mls. with distilled water. For standardization see page 200.[2]

[1] The astringent principle of tea, etc. Gallic acid, 
; tannic acid, 
probably united with glucose or with phloroglucinol.

[2] There is confusion concerning the term "hypo" which is sometimes assigned to this substance. The term "hypo" is used in photography to designate this product,

Trichloracetic Acid, CCl_3COOH.—50 gs. of the crystals are rapidly weighed on a rough balance and made up to 1000 mls. of distilled water solution. This makes a 5 per cent. solution.

Trypsin: Method of Preparation from Pancreas.—In asking for pancreas at the abbatoir be sure to specify the belly-gland, known to German butchers as "Bauspeichel-druese." The thymus from the calf is generally sold on the retail market as "sweet-bread." By means of scissors remove the fat as much as possible from the gland. Mince the gland substance and add 300 mls. of 95 per cent. ethanol. Add about 5 mls. of toluene and an equal amount of chloroform. Shake the bottle and leave in a cool place for several days. When ready to use the extract, pour off the liquid and use for trypsinogen experiments and, for active trypsin, add the juice of some of the mucosa of the duodenum, or jejunum, which contains enterokinase. The solution must not be filtered if the extract is to be used for fat digestion experiments. For trypsin alone preserve by filtering through paper and adding to the filtrate a small amount of concentrated HCl (about 1 ml. per liter of filtrate).

Turpentine for Demonstrating Hematuria in Experimental Animals.—By mouth to dog, 20 gs. of turpentine oil.

Uffelmann's Reagent.—Treat a 2 per cent. solution of phenol with 0.5 per cent. ferric chlorid solution until the solution becomes colored a purplish violet.

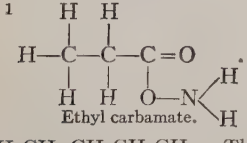
Urea, as diuretic, 15 gs. urea crystals administered in a small amount of water. In order to mask the taste add a little honey to the crystals and mix well by means of a spatula. The dose may be repeated up to total of 40 gs. per day.

Urease Paper (Folin).—Shake 15 gms. of powdered meal with 10 gms. of permutit and 100 mls. of 15 per cent. ethanol in a 500 ml. flask for 30 mins. Filter through several filters into a large evaporating dish. Saturate long strips of good quality filter paper and hang up to dry. This paper keeps indefinitely in a dry and dark place. Use a half inch square for each determination.

Urease Solution.—Grind 5 gs. of soy-bean or jack-bean, whole-bean or meal, with 50 mls. of 0.6 per cent. KH_2PO_4. Let stand about fifteen minutes and then filter. Keep in a cool place. The solution remains active for about two days. A more stable solution is obtained as follows (after Folin): Weigh roughly 5 gs. of powdered soy- or jack-bean meal into a 500-ml. Erlenmeyer flask. Add freshly acid washed permutit powder (3 gs.) and 85 mls. of distilled water; add 16 mls. of 95 per cent. ethanol. Agitate the contents of the flask for a quarter of an hour and filter. The solution thus prepared will be active for a week at 20° C., or longer if kept at ice-box temperature (about 8° C.). Urease tablets (Sumner): Grind about 50 gs. of jack-beans in a meat-grinder with coarse grinder and pass the ground beans through again, using the finer grinder. Then transfer to a large mortar and grind as fine as possible. Strain through a 20-mesh brass sieve, collecting the siftings in a large mortar. Moisten the siftings with a little 95 per cent. alcohol. If a tablet-mould is available, press the moistened powder into pellets of about 60 mgs. each. Let the pellets dry in the mould. Store the tablets in a well-stoppered bottle. Time for completely deaminizing urea in 1 ml. of urine twenty minutes at 20° C.

Urethan, for anesthesia in experimental animal.[1] Rabbit: 1 g. per kilo, by stomach-tube.

$Na_2S_2O_3.5H_2O$, but in some quarters it is applied to sodium hyposulphite, $Na_2S_2O_4$, also called hydrosulphite.

[1] 
Ethyl carbamate.

. Other urethanes are $NH(C_6H_5)CO.O.C_2H_5$, $NH_2CO.O.CH.CH_3.CH_2CH_2CH_3$.
Phenyl urethan. Hedonal.

The one referred to in the text is the first, ethyl carbamate.

TABLE GIVING WEIGHTS (MGS.) OF NH_2 NITROGEN PER ML. OF NITROGEN GAS AT GIVEN TEMPERATURES (C. °) AND PRESSURES (MMS.)

After Van Slyke, Jour. Biol. Chem., vol. 12, page 275, 1912.

t	728	730	732	734	736	738	740	742	744	746	748	750
11°	0·5680	0·5695	0·5510	0·5725	0·5745	0·5760	0·5775	0·5790	0·5805	0·5820	0·5840	0·5855
12°	0·5655	0·5670	0·5685	0·5700	0·5720	0·5735	0·5750	0·5765	0·5780	0·5795	0·5815	0·5830
13°	0·5630	0·5645	0·5660	0·5675	0·5695	0·5710	0·5725	0·5740	0·5755	0·5770	0·5785	0·5805
14°	0·5605	0·5620	0·5635	0·5650	0·5665	0·5680	0·5700	0·5715	0·5730	0·5745	0·5760	0·5775
15°	0·5580	0·5595	0·5610	0·5625	0·5640	0·5655	0·5670	0·5685	0·5705	0·5720	0·5735	0·5750
16°	0·5555	0·5570	0·5585	0·5600	0·5615	0·5630	0·5645	0·5660	0·5675	0·5690	0·5710	0·5725
17°	0·5525	0·5540	0·5555	0·5575	0·5590	0·5605	0·5620	0·5635	0·5650	0·5665	0·5680	0·5695
18°	0·5500	0·5515	0·5530	0·5545	0·5560	0·5580	0·5595	0·5610	0·5625	0·5640	0·5655	0·5670
19°	0·5475	0·5490	0·5505	0·5520	0·5535	0·5550	0·5565	0·5580	0·5595	0·5610	0·5630	0·5645
20°	0·5445	0·5460	0·5475	0·5495	0·5510	0·5525	0·5540	0·5555	0·5570	0·5585	0·5600	0·5615
21°	0·5420	0·5435	0·5450	0·5465	0·5480	0·5495	0·5510	0·5525	0·5540	0·5555	0·5575	0·5590
22°	0·5395	0·5410	0·5425	0·5440	0·5455	0·5470	0·5485	0·5500	0·5515	0·5530	0·5545	0·5560
23°	0·5365	0·5380	0·5395	0·5410	0·5425	0·5440	0·5455	0·5470	0·5485	0·5500	0·5515	0·5530
24°	0·5335	0·5350	0·5365	0·5380	0·5400	0·5415	0·5430	0·5445	0·5460	0·5475	0·5490	0·5505
25°	0·5310	0·5325	0·5340	0·5355	0·5370	0·5385	0·5400	0·5415	0·5430	0·5445	0·5460	0·5475
26°	0·5260	0·5295	0·5310	0·5325	0·5340	0·5355	0·5365	0·5370	0·5400	0·5415	0·5430	0·5445
27°	0·5250	0·5265	0·5280	0·5295	0·5310	0·5325	0·5340	0·5355	0·5370	0·5385	0·5400	0·5415
28°	0·5220	0·5235	0·5250	0·5265	0·5280	0·5295	0·5310	0·5325	0·5340	0·5355	0·5370	0·5385
29°	0·5195	0·5210	0·5220	0·5235	0·5250	0·5265	0·5280	0·5295	0·5310	0·5325	0·5340	0·5355
30°	0·5160	0·5175	0·5190	0·5205	0·5220	0·5235	0·5250	0·5265	0·5280	0·5295	0·5310	0·5325

t	752	754	756	758	760	762	764	766	768	770	772
11°	0·5870	0·5885	0·5900	0·5915	0·5935	0·5950	0·5965	0·5980	0·5995	0·6010	0·6030
12°	0·5845	0·5860	0·5875	0·5890	0·5905	0·5925	0·5940	0·5955	0·5970	0·5985	0·6000
13°	0·5820	0·5835	0·5850	0·5865	0·5880	0·5895	0·5910	0·5930	0·5945	0·5960	0·5975
14°	0·5790	0·5805	0·5825	0·5840	0·5855	0·5870	0·5885	0·5900	0·5915	0·5935	0·5950
15°	0·5765	0·5765	0·5795	0·5810	0·5830	0·5845	0·5860	0·5875	0·5890	0·5905	0·5920
16°	0·5740	0·5755	0·5770	0·5785	0·5800	0·5815	0·5830	0·5850	0·5865	0·5880	0·5895
17°	0·5710	0·5730	0·5745	0·5760	0·5775	0·5790	0·5805	0·5820	0·5825	0·5850	0·5865
18°	0·5685	0·5700	0·5715	0·5730	0·5745	0·5765	0·5780	0·5795	0·5810	0·5825	0·5840
19°	0·5660	0·5675	0·5690	0·5705	0·5720	0·5735	0·5750	0·5765	0·5780	0·5795	0·5810
20°	0·5630	0·5645	0·5660	0·5675	0·5690	0·5705	0·5725	0·5740	0·5755	0·5770	0·5785
21°	0·5605	0·5620	0·5635	0·5650	0·5665	0·5680	0·5695	0·5710	0·5725	0·5740	0·5755
22°	0·5575	0·5590	0·5605	0·5620	0·5635	0·5650	0·5665	0·5680	0·5695	0·5715	0·5730
23°	0·5545	0·5560	0·5575	0·5595	0·5610	0·5625	0·5640	0·5655	0·5670	0·5685	0·5700
24°	0·5520	0·5535	0·5550	0·5565	0·5580	0·5595	0·5610	0·5625	0·5640	0·5655	0·5670
25°	0·5490	0·5505	0·5520	0·5535	0·5550	0·5565	0·5580	0·5595	0·5610	0·5625	0·5640
26°	0·5460	0·5475	0·5490	0·5505	0·5520	0·5535	0·5550	0·5565	0·5580	0·5595	0·5610
27°	0·5430	0·5445	0·5460	0·5475	0·5490	0·5505	0·5520	0·5535	0·5550	0·5565	0·5580
28°	0·5400	0·5415	0·5430	0·5445	0·5460	0·5475	0·5490	0·5505	0·5520	0·5535	0·5550
29°	0·5370	0·5385	0·5400	0·5415	0·5430	0·5445	0·5460	0·5475	0·5490	0·5505	0·5520
30°	0·5340	0·5355	0·5370	0·5385	0·5400	0·5415	0·5430	0·5445	0·5460	0·5475	0·5490

Uranium Acetate.—Normal solution: Weigh 21.30 gs. of the crystal ($UrO_2(C_2H_3O_2)_2.H_2O$) and make up to 1000 mls. with distilled water.

Uranium Nitrate.—Decinormal solution: Weigh 25.18 gs. of uranium nitrate crystals, ($UrO_2(NO_3)_2.6H_2O$), and make up to 1000 mls. with H_2O. Sodium acetate must be used with phosphorus analyses in order to prevent the formation of free nitric acid.

Uranium Salts for Experimental Nephritis in Animals.—Dog: Hypodermic injection of 2 to 15 mgs. per kilo. Fatal dose, 1.66 gs. (Sollmann).

Van Slyke's Aceton Solutions.—See page 888.

Van Slyke's Oxygen Solutions.—(Volumetric method; Fig. 36, page 77.) Capacity measurements: (1) Saponin,[1] to cause hemolysis of the erythrocytes, 1 per cent. solution, aqueous.

(2) Caprylic alcohol, $C_8H_{15}OH$, antifoaming agent.

(3) Potassium ferricyanid solution; 20 gs. up to 100 mls. H_2O.

(4) Sodium hydroxid, 0.5 normal solution: Dilute normal with 1 volume of H_2O.

Oxygen content: (1) Hemoglobin standard: Acid hematin from standard hemoglobin derived from oxygen capacity measurements: Determine the oxygen capacity by the method using the above reagents (page 389) and multiply the figure representing the oxygen combined with 100 mls. of blood by the factor 0.746; this gives the grams of hemoglobin per 100 mls. of blood. From such blood make an acid hematin solution by adding as much blood as necessary to a decinormal hydrochloric acid solution as will make a 3.75 per cent. hemoglobin solution. It is necessary to observe certain corrections in this calculation since we are dealing with gases:

Temperature at which determination is made:	Factor converting the burette reading to standard temperature and pressure.
15° C.	$0.932 \times \frac{\text{barometric reading}}{760}$
16° C.	.928
17° C.	.924
18° C.	.919
19° C.	.915
20° C.	.910
21° C.	.906
22° C.	.901
23° C.	.897
24° C.	.892
25° C.	.888
26° C.	.883
27° C.	.878
28° C.	.873
29° C.	.868
30° C.	.863

Multiply the observed gas volume (reading on the burette) by the proper factor in the second column × the fraction following that factor $\left(\frac{B}{760}\right)$ and then by the special figure indicating the volume of blood taken for analysis (100 for 1 ml.; 50, for 2 mls., etc.). Finally, subtract 1.36, the volume per cent. nitrogen which accom-

[1] The term "saponin" is a group name for substances of the nature of glucosides and galactosides found in plants. The common commercial saponin is derived from the Soap-weed, Saponaria.

panies the gases, but is inert. The grams of hemoglobin in 100 mls. of blood = 0.746 × O_2 bound by blood saturated with air at room temperature (20° C.). This last figure is obtained by subtracting 2.1 from the result obtained after multiplying by 100 or 50, etc., (page 929).

If the Newcomer glass colorimetric standard is used, it is equivalent to using an acid hematin standard 0.038 g. hemoglobin per 100 mls. blood.

From the standard acid hematin solution make by suitable dilution, a working standard containing 0.075 g. hemoglobin per 100 mls. blood is obtained.

Methemoglobin estimation: Stadie's solutions:

Potassium ferricyanid, 3 per cent. solution in water.

Potassium cyanid, 0.1 per cent. solution in water.

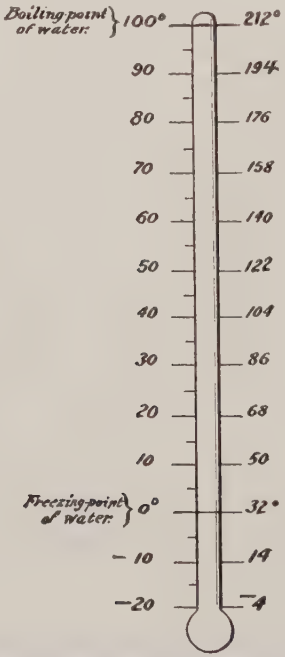
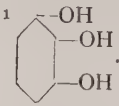


Fig. 272.—Thermometer showing Centigrade and Fahrenheit scales. "Absolute zero," −273° C.

Carbon monoxid solutions (Van Slyke and Salvesen):

Solutions are in part the same as those used in the determination of oxygen capacity. In addition: Pyrogallol solution: 10 gs. of pyrogallol[1] in 200 mls. of a solution of KOH, made by dissolving 160 gs. KOH in 130 mls. of H_2O.

Reagents for the manometric method by Van Slyke (page 393): (1) Sodium hydrosulphite solution, $Na_2S_2O_4.2H_2O$: Weigh 10 gs. and add 50 mls. of half-normal KOH solution; stir by means of a stirring-rod. Filter through glass-wool or cotton. Render the liquid gas free by subjecting it to the vacuum of the apparatus.[2]

[1] 

. Pyrogallol is the same as pyrogallic acid.

[2] See description of method, page 393.

(2) Pyrogallol may replace the hydrosulphite (2) in the absorption of oxygen. This reagent is given above. It is unnecessary to extract this solution.

(3) This reagent is virtually a combination of those described for the Van Slyke and Stadie method above. Single solution:

Potassium ferricyanid	3.0 gs.
Saponin (Merck's)	3.0 gs.
Octyl alcohol	3.0 mls.
Water to make	1000.0 mls.

(4) For CO determinations. Single solution:

Saponin (Merck's)	3.0 gs.
Potassium ferricyanid	8.0 gs.
Lactic acid, normal solution	40.0 mls.
Caprylic alcohol	3.0 mls.
Water to make	1000.0 mls.

(5) Special reagents for freeing the blood of gases:

For O_2 determinations (as described above).

For CO_2, 0.017 n. lactic acid made by diluting 1 ml. of the normal lactic acid to 60 mls. solution.

For combined CO_2 and O_2 the neutral ferricyanid solution described under (2) above, but having 1 ml. of normal lactic acid per 60 mls. of solution.

Vernier.—This is a means of dividing any unit measurement into decimal parts. A movable slide bears a graduated strip in tenths, in such a manner that it may be applied to any unit, as, for instance, the millimeter marks on the colorimeter. The zero of the vernier marks the unit to be read and the coinciding lines mark the decimals (tenths) to be added to the reading. Thus, if the zero of the vernier fall upon the interval 8.0 to 9.0 mms. of the colorimeter and it is found that the line representing 4 on the vernier coincides with a line on the millimeter scale, then the reading is 8.4 mms.

Van Slyke Reagents for Gasometric Amino-nitrogen Method.—Sodium nitrite solution:

Sodium nitrite sticks, $NaNO_2$	30 gs.
Water up to	100 mls.

Acetic acid solution: Glacial acetic acid.

Solution for Hempel pipette:

Potassium permanganate, $KMnO_4$	40 gs.
Potassium hydroxid, KOH (or NaOH)	25 gs.
Water to make	1000 mls.

Volhard's Chlorid Reagents—0.1 *N. Silver Nitrate.*—Dissolve 16.99 gs. of pure fused silver nitrate in distilled water and make the volume up to 1 liter. Keep the solution in the dark.

$$1 \text{ ml.} = 0.00365 \text{ g. HCl.}$$

0.1 *N. Thiocyanate.*—Dissolve about 15 gs. of potassium, or about 10 gs. of ammonium thiocyanate, in a liter of distilled water. Mix thoroughly. Standardize this in the following way: Pipette 20 mls. of the standard silver nitrate into a 150-ml. beaker or Erlenmeyer flask. Add about 60 mls. of distilled water, 5 mls. of pure concentrated nitric acid, and 5 mls. of a cold saturated solution of iron alum (ferric ammonium sulphate). Titrate with the thiocyanate from a burette. A white precipitate of silver thiocyanate is formed. Continue to add the thiocyanate until a faint permanent pink (due to ferric thiocyanate) is obtained. Let x mls. = thiocyanate required. To 1 liter of the solution add $\frac{1000\ (20 - x)}{x}$ mls. of distilled water.

Wijs' Solution.—(1) Original method: Dissolve, in a liter of glacial acetic acid, 13 gs. of resublimed iodin. Determine the strength of the iodin solution by titrating with standard thiosulphate solution.[1] Run in this thiosulphate from a burette until the iodin solution becomes light yellow; then add starch solution and continue adding the thiosulphate until a blue color just disappears.

(2) Dissolve 9.4 gs. iodin trichlorid in 200 mls. glacial acetic acid. Heat carefully over a gauze and stopper the flask with a cork stopper bearing a $CaCl_2$ tube. Dissolve 7.2 gs. resublimed iodin in another flask containing 200 mls. glacial acetic acid, as before. Pour the two solutions into a liter volumetric flask. Cool. Make up to the mark with glacial acetic acid. Titrate this solution with decinormal thiosulphate as in (1) above.

PIPETTE TECHNIQUE[2]

Transfer[3] and *Measuring Pipettes.*[4]—In filling pipettes the liquid should be drawn to approximately 10 mms. above the zero (0) and then slowly emptied until the liquid is correctly set with the bottom of the meniscus at the zero mark. Any liquid adhering to the tip should be removed with filter-paper after the setting of the zero.

To empty a transfer pipette it should be held in a vertical position; the outflow should be unrestricted until the surface of the water reaches the upper end of the delivery tube. The tip should then be touched to the wet surface of the receiving vessel and kept in contact with it until the emptying is complete. *The water remaining in the tip should not be blown out.*

To empty a measuring pipette it should be held in a vertical position and continuous, unrestricted outflow permitted until the liquid approaches the graduation mark on pipette. It is necessary to arrest the full flow of the liquid in time to obtain control over the final movement of the water surface and to bring the meniscus to rest gradually on the line to be tested. The instrument, therefore, is allowed to deliver freely until the water surface is approximately 10 mms. from the line representing the volume to be discharged. The rate of outflow is then reduced and the motion of the water surface brought under control so that any accurate setting can be made on the line in question. No period of waiting for drainage is allowed. The liquid adhering to the outside of the tip after the setting is then removed by touching it to the wet surface of the receiving vessel, but no liquid from within the pipette may be permitted to leave it.

[1] The standardization is carried out as follows: Dissolve exactly 3.863 gs. crystal potassium dichromate in distilled water and make up to 1000 mls. After dissolution of the crystals, pipette 20 mls. into a 200-ml. bottle, add 25 mls. water, then 10 mls. 10 per cent. potassium iodid solution. Finally add 5 mls. of pure hydrochloric acid. Stopper and leave in a closet five minutes. The solution contains exactly 0.2 g. iodin, set free according to the following reaction:

$$K_2Cr_2O_7 + 14HCl + 6KI = 8KCl + Cr_2Cl_6 + 7H_2O + 3I_2;$$
$$3I_2 + 6Na_2S_2O_3 = 6NaI + 3Na_2S_4O_6.$$

[2] *References.*—For the data concerning the proper use of pipettes used in this book the author is greatly indebted to the firm of Arthur H. Thomas Co., in the persons of Mr. Thomas and Mr. Patterson. Reference may be made to:

Bureau of Standards Circular, No. 9, Testing of Glass Volumetric Apparatus.

Glazebrook's Dictionary of Applied Physics, Vol. 3, Measurements of Volume.

The National Physical Laboratory, November, 1919, Volumetric Tests on Scientific Glassware.

[3] Such pipettes as the ordinary volumetric pipettes with only one graduation. Also called "tip-off" pipettes.

[4] The Mohr pipette belongs in this group.

Ostwald-Folin Pipettes—"Contain" Pipettes.—The filling and emptying of the Serological Pipettes graduated to the tip and Ostwald-Folin Pipettes to deliver are the same as described for the transfer pipette, excepting the remaining drop in the end of the tube is removed by blowing through the pipette, or follow the directions on page 66, where the warmth of the hand is used to force out the remaining drops. Because breath contains CO_2 care should be taken with pipettes.

Contain Pipettes.—Pipettes to contain are used the same way as Transfer Pipettes, but the pipette is rinsed out so as to insure the complete delivery of its contents.

LOGARITHMS

are used to abbreviate the time and the number of stages in calculations. The kind of logarithm used in most chemical calculations is the common or Brigg's logarithm which may be defined as the number indicating how many times 10 is to be multiplied by itself to give the number sought.

Ten multiplied by itself no times $= 10^0$; the log is 0.0.
Ten multiplied by itself once $= 10^1$; the log is 1.0.
Ten multiplied by itself twice $= 10^2$; the log is 2.0.

When the log is ^{0}, the number is 1; when it is $^{-1}$, the number is 0.1.
When the log is ^{1}, the number is 10; when it is $^{-2}$, the number is .01.
When the log is ^{2}, the number is 100; when it is $^{-3}$, the number is .001.

Numbers between 1 and 10 have fractions as logs:

$2 = 10^{0.3010}$ Log of 2 is 0.3010.
$3 = 10^{.4771}$ Log of 3 is .4771.
$4 = 10^{.6021}$ Log of 4 is .6021.

Now it is possible to represent numbers by lines of certain lengths; thus we may say that 2 is |——|; 3 is |———|. In like manner, we may say that 0.3010 is |—|; that 0.6021 is |—|. The scales C and D on a slide rule are lines representing the logarithms of numbers from 1 to 10, which may likewise be used to represent any such relationship, like 10 to 100; 100 to 1000, etc. The lines are not all alike; the length of the line from 1 to 2 is much longer than that from 2 to 3, etc. This makes no difference in the use of the rule for multiplying and dividing, because all that we wish the rule to do is to add and to subtract; for when logs are added, the numbers they represent are multiplied:

$$\text{Log } 2 + \log 2 = \log 4 = 10{,}000$$
$$\text{Log } 2 = 100;\ \log 2 = 100;\ 100 \times 100 = 10{,}000$$

When logs are subtracted the numbers are divided:

$$\text{Log } 4 - \log 2 = \log 2 = 100$$
$$\text{Log } 4 = 10{,}000;\ \log 2 = 100;\ 10{,}000 \div 100 = 100$$

As the rule is set in Fig. 242, page 823, we are adding log 10.5 to every log on the scale Ur. that is in contact with scale E; in other words, we are multiplying the numbers corresponding to these logs. If we multiply by the scale C turned the opposite way (as in the scale CI, Fig. 14), we are dividing because: $2 \times 4 = 8$; $\frac{1}{2} \times 4 = 2$.

Slide-rule:[1] This is a convenient method for working with logarithms. If two numbers are to be multiplied, their logs are added; if they are to be divided, their logs are to be subtracted. The slide-rule simply adds or subtracts logarithms.

[1] The directions are for the Keuffel and Esser duplex type, Fig. 14, page 42. The Log-log-rule made by this firm permits direct readings of logs and interconversions of common (Brigg's) and natural (Naperian) logs. See p. 61.

Rules for decimal points with Duplex rule:

Multiplication: Slide to	left, sum of integers		Black.
	right, sum $-$ 1.		
Division: Slide to	left, difference $+$ 1		Red.
	right, difference.		

Hydrogen-ion and *p*H conversions by the slide rule: Example: Convert 2.88 $(10)^{-10}$ to *p*H. Find on the scale CI, 288, and read on the scale L, beneath 288, the figures 54. Place a decimal point in front of these numbers, making 0.54. Write in front of the decimal point the figure 9, making the completed *p*H = 9.54. The figure 9 is used because it is one less than the exponent $^{-10}$ of (10). The procedure is reversed when passing from *p*H to C_H.

SPECIAL TABLES

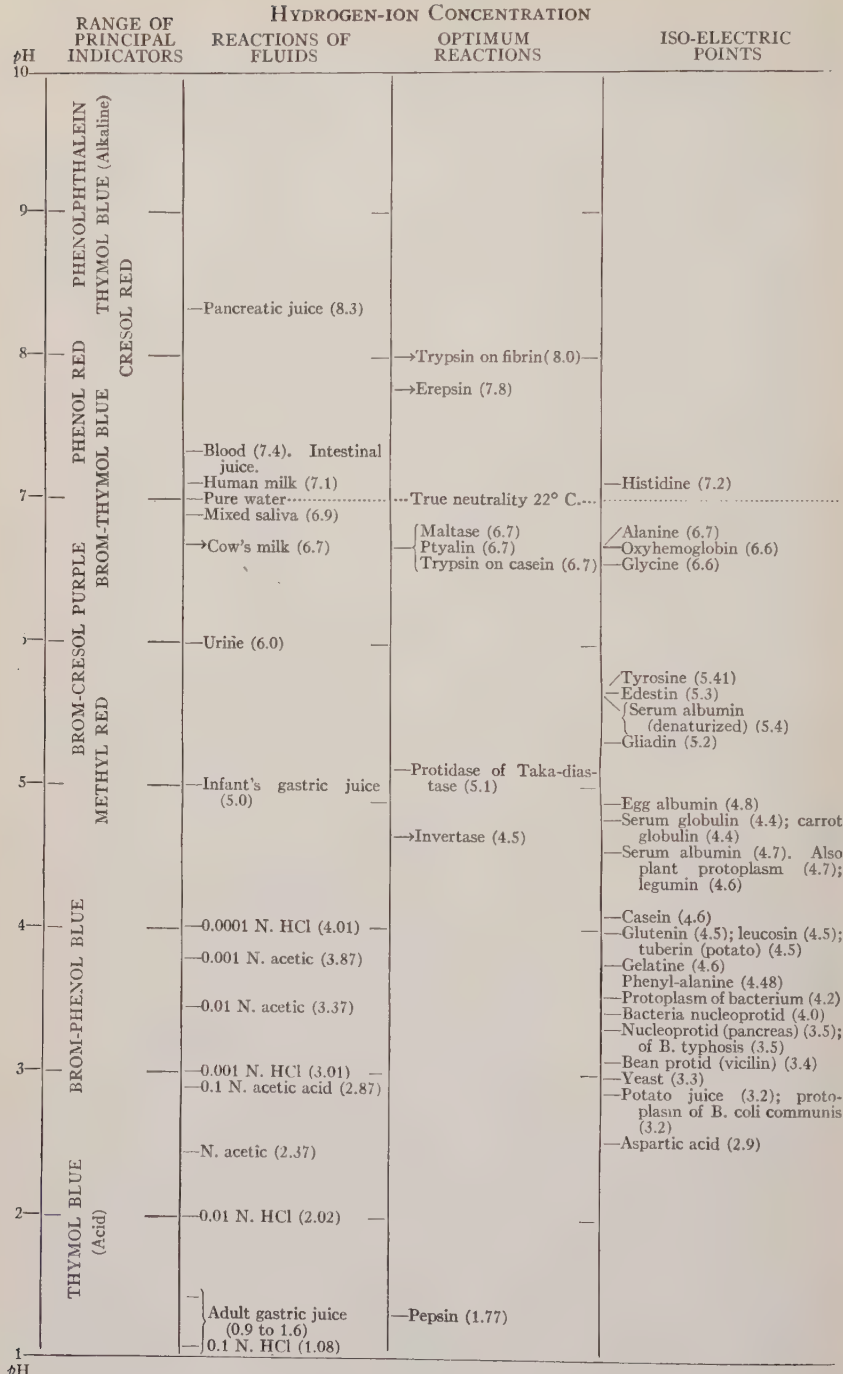
TABLE SHOWING THE WEIGHT OF 1 ML. OF WATER AT DIFFERENT TEMPERATURES. THE FIGURES ARE BASED ON WEIGHINGS WITH BRASS WEIGHTS IN AIR

Temperature, degrees Centigrade.	Weight of 1 ml. of water in gs.	Volume of 1 g. of water in mls.
15	0.9981	1.0019
16	0.9979	1.0021
17	0.9977	1.0023
18	0.9976	1.0024
19	0.9974	1.0026
20	0.9972	1.0028
21	0.9970	1.0030
22	0.9967	1.0033
23	0.9965	1.0035
24	0.9963	1.0037
25	0.9960	1.0040
26	0.9958	1.0042
27	0.9955	1.0045
28	0.9952	1.0048
29	0.9949	1.0051
30	0.9946	1.0054
31	0.9943	1.0057
32	0.9941	1.0055

TABLE SHOWING THE VARIATIONS IN THE VALUE OF GRAVITY IN DIFFERENT EDUCATIONAL CENTERS. THE VALUE VARIES WITH LATITUDE AND WITH ALTITUDE

GRAVITY CONSTANTS

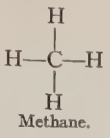
	G. per cm. per sec.	
Atlanta, Ga	979.5	(Emory University).
Austin, Texas	979.2	(at the University of Texas).
Baltimore, Md	980.09	(Johns Hopkins Hospital).
Cambridge, Mass	980.39	(Harvard University).
Charlottesville	979.93	(University of Virginia).
Chicago, Ill	980.27	(University of Chicago).
Cincinnati, O	980.00	(University of Cincinnati).
Cleveland, O	980.24	(Western Reserve University).
Denver, Colo	979.68	(University of Colorado, Denver).
Galveston, Tex	979.27	(University of Texas).
Ithaca, N. Y	980.29	(Cornell University).
Little Rock, Ark	979.72	(University of Arkansas).
Madison, Wis	980.36	(University of Wisconsin).
New Orleans, La	979.32	(Tulane University).
New York, N. Y	980.26	(Columbia University, Morningside).
Philadelphia, Pa	980.15	(University of Pennsylvania).
Princeton, N. J	980.17	(Princeton University).
Salt Lake City	979.80	(University of Utah).
San Francisco	979.95	(University of California; Stanford University).
St. Louis, Mo	980.00	(Washington University; St. Louis University).
Washington, D. C	980.11	(Smithsonian Institution, etc.).
Worcester, Mass	980.32	(Clark University).

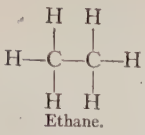

HYDROGEN-ION CONCENTRATION
RANGE OF PRINCIPAL INDICATORS
REACTIONS OF FLUIDS
OPTIMUM REACTIONS
ISO-ELECTRIC POINTS
pH
10
9
8
7
6
5
4
3
2
1
pH
PHENOLPHTHALEIN
THYMOL BLUE (Alkaline)
CRESOL RED
PHENOL RED
BROM-THYMOL BLUE
BROM-CRESOL PURPLE
METHYL RED
BROM-PHENOL BLUE
THYMOL BLUE (Acid)
—Pancreatic juice (8.3)
—Blood (7.4). Intestinal juice.
—Human milk (7.1)
—Pure water
—Mixed saliva (6.9)
→Cow's milk (6.7)
—Urine (6.0)
—Infant's gastric juice (5.0)
—0.0001 N. HCl (4.01)
—0.001 N. acetic (3.87)
—0.01 N. acetic (3.37)
—0.001 N. HCl (3.01)
—0.1 N. acetic acid (2.87)
—N. acetic (2.37)
—0.01 N. HCl (2.02)
Adult gastric juice (0.9 to 1.6)
—0.1 N. HCl (1.08)
→Trypsin on fibrin (8.0)
→Erepsin (7.8)
...True neutrality 22° C....
Maltase (6.7)
Ptyalin (6.7)
Trypsin on casein (6.7)
—Protidase of Taka-diastase (5.1)
→Invertase (4.5)
—Pepsin (1.77)
—Histidine (7.2)
—Alanine (6.7)
—Oxyhemoglobin (6.6)
—Glycine (6.6)
—Tyrosine (5.41)
—Edestin (5.3)
—Serum albumin (denaturized) (5.4)
—Gliadin (5.2)
—Egg albumin (4.8)
—Serum globulin (4.4); carrot globulin (4.4)
—Serum albumin (4.7). Also plant protoplasm (4.7); legumin (4.6)
—Casein (4.6)
—Glutenin (4.5); leucosin (4.5); tuberin (potato) (4.5)
—Gelatine (4.6)
Phenyl-alanine (4.48)
—Protoplasm of bacterium (4.2)
—Bacteria nucleoprotid (4.0)
—Nucleoprotid (pancreas) (3.5); of B. typhosis (3.5)
—Bean protid (vicilin) (3.4)
—Yeast (3.3)
—Potato juice (3.2); protoplasm of B. coli communis (3.2)
—Aspartic acid (2.9)

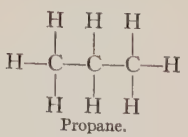
CHARACTERISTIC BIOCHEMICAL CONFIGURATIONS

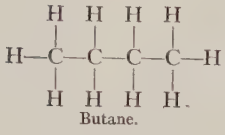
Hydrocarbon series.

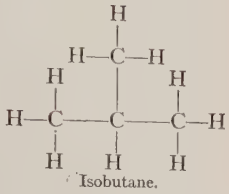
Saturated:


Methane.

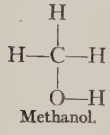

Ethane.

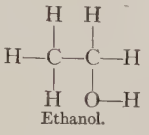

Propane.

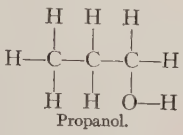

Butane.


Isobutane.

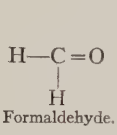
Alcohols

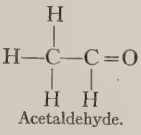

Methanol.


Ethanol.

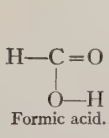

Propanol.

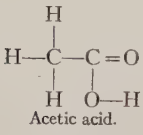
Aldehydes

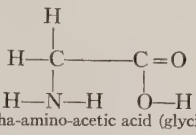

Formaldehyde.


Acetaldehyde.

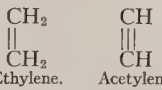
Acids

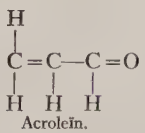

Formic acid.

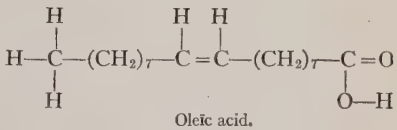

Acetic acid.


Alpha-amino-acetic acid (glycin).

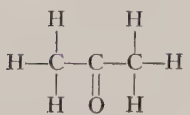
Unsaturated:

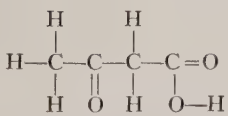

Ethylene. Acetylene.


Acroleïn.

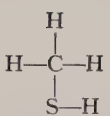

Oleïc acid.

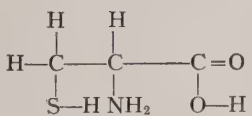
Ketones


Aceton.


Di-acetic acid.

Mercaptans
(thio-alcohols)

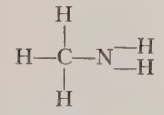

Methyl mercaptan.


Alpha-amino-thiopropionic acid.

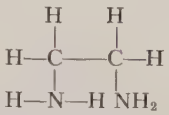
ALCOHOLS, ALDEHYDES, AND ACIDS

Saturated hydrocarbon.	Primary alcohol, —CH_2OH.	Aldehyd —CHO.	Fatty acid, —COOH.	Carbon atoms.
Methane	Methyl	Formaldehyde	Formic	1
Ethane	Ethyl	Acetaldehyde	Acetic	2
Propane	Propyl	Propionic aldehyde	Propionic	3
Butane	Butyl	Butyric "	Butyric	4
Pentane	Amyl	Valeric "	Valerianic	5
Hexane	Hexyl	Caproic "	Capronïc	6
Heptane	Heptyl	Enanthic "	Enanthic	7
Octane	Octyl	Caprylic "	Caprylic	8
Nonane	Nonyl	Pelargonic "	Pelargonic	9
Decane	Decyl	Capric "	Caprinic	10
Undecane	Undecyl	Undecylic "	Undecylic	11
Dodecane	Dodecyl	Lauric "	Lauric	12
Tridecane	——	Tridecyclic "	Tridecylic	13
Tetradecane	Tetradecyl	Myristic "	Myristic	14
Pentadecane	——	——	Pentadecylic	15
Hexadecane	Cetyl	Palmitic "	Palmitic	16
Heptadecane	——	Margaric "	Margaric; lignoceric	17
Octadecane	Octadecyl	Stearic "	Stearic; ricinoleïc	18
Nonadecane	——	——	——	19
Eicosane	——	——	Arachidonic	20
Heneicosane	——	——	——	21
Docosane	——	——	Behenic	22
Tricosane	——	——	——	23
Tetracosane	——	——	Lignoceric	24
——	——	——	——	25
——	Ceryl	——	Cerotic	26
Heptacosane	——	——	——	27
——	——	——	——	28
——	——	——	——	29
——	Myricyl	——	Melissic	30

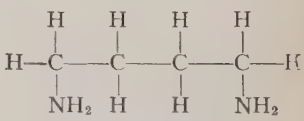
Amins:



Primary amin, mono-methylamin.

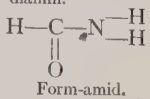


Di-amin, ethylene-diamin.



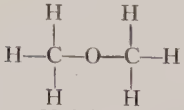
Di-amin, "putrescin," butylel-diamin.

Amids:

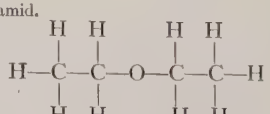


Form-amid.

Ethers:

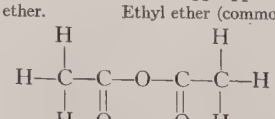


Methyl ether.



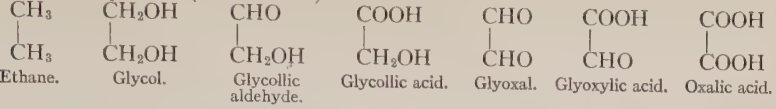
Ethyl ether (common "sulphuric" ether).

Anhydrides:

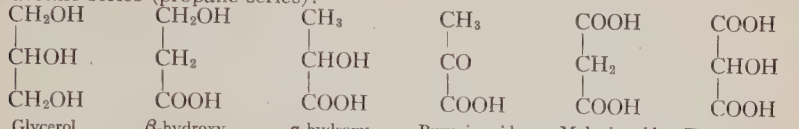


Acetic anhydride.

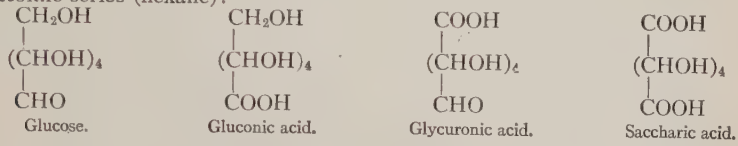
Di-atomic compounds (ethan series):



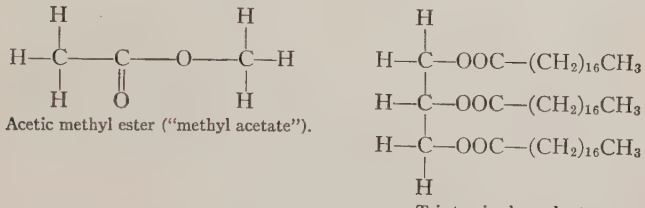
Tri-atomic series (propane series):



Hex-atomic series (hexane):



Esters:



SCHEME FOR THE ANALYSIS OF MILK[1]

Total Butter Fat.—See page 411. The "lactocrite" method of Leffmann and Beam (Analysis of Milk and Milk Products, Blakiston, Philadelphia) antedates Babcock's method and is similar to it.

Lactose.—Myers' (German chemist) method: Precipitate 1 volume of carefully mixed milk with 1 volume of the special acid solution;[2] then dilute with 2 volumes of distilled water. Mix. Filter, repeating the filtering through the same paper until the filtrate is clear. Place the filtrate (or part) in a burette and follow the quantitative procedure of Folin (page 777), or of Benedict (page 44), using the figure 67 in place of 50 mgs. for lactose with Benedict's solution.[3]

Dialyzed iron (page 105) may be used to replace the phosphotungstic HCl solution. Dilute the iron solution with 10 volumes of water and use of this solution 5 mls. to each 10 mls. of milk diluted with about one volume of distilled water.

For Folin and Denis' method see page 418. This method uses milk directly without previous precipitation.

[1] For chemical examination of human milk, the specimen must be taken from the breast without undue pressure. The container must be stoppered with cork or rubber stopper and never with cotton, because cotton absorbs the cream which accumulates when the specimen stands.

[2] Place 200 mls. concentrated hydrochloric acid in a liter volumetric flask and add an equal volume of distilled water. Add 70 gs. phosphotungstic acid ($P_2O_5.WO_3 + H_2O$), mix, and make up to a liter.

[3] If Fehling's Method (page 160) is used, the Cu in 10 mls. is reduced by 67.6 mgs. lactose.

Total Protid.—For small amounts of milk, such as the clinical laboratory frequently receives for analyses, proceed as follows:

Mix the sample thoroughly and then pipette (Ostwald-Folin 1-ml. pipette) 1 ml. into a 25-ml. volumetric flask. Add 1 drop of 10 per cent. NaOH solution, or a knife-point of powdered lithium carbonate in order to hold the casein in suspension. Add about 10 mls. of distilled water and mix thoroughly. Then dilute to the mark, adding a drop of caprylic alcohol if necessary to cut down foam. Transfer the diluted milk to a small beaker and pipette 2 mls. of the well-mixed specimen into a 200 × 20 mm. pyrex test-tube. Follow thence forward the method for nitrogen determination of Folin given on page 698.[1] In making the calculation remember the dilution that you have made.

If desired, a larger amount of milk (5 mls.) may be used and the Kjeldahl method (page 697) may be employed.

Total Solids.—Carefully clean a small (8 cm. diameter) evaporating dish and heat it to redness over an asbestos gauze with a blue flame in order to avoid deposition of soot. Let cool, preferably in a desiccator (page 93), and then weigh to the second place of decimals. Then pipette by means of the Ostwald-Folin pipette, 1 ml. of well-mixed milk into the dish and smear it over the inner surface of the dish. Leave the dish in the thermostat at 40° C., or on a steam-bath protected from dust by means of another evaporating dish inverted over the first one and slightly raised by means of small pieces of cork stoppers. At the end of the drying period (about two hours) remove to a desiccator and leave until cool. Then reweigh. The difference in weights gives the water evaporated and the remainder the total solids. Ash may be determined by a similar procedure, using a small porcelain, nickel, silver, or platinum crucible, 5 mls. of milk, and evaporating rapidly over an open flame, the crucible being suspended on a pipe-stem triangle. Care must be taken that no sputtering occur, thus losing the fluid. Follow the directions given above for total solids, weighing being carried to the fourth place of decimals.

As in the case of urine, there is a correlation between certain constituents and total solids. The Hehner-Richman formula connecting specific gravity, fat, and total solids is as follows:

$$\text{Total fat by } \frac{\text{Babcock}}{\text{Lactocrite}} \text{ method} = 0.859 \times \text{solids} - 0.2186 \times \text{specific gravity.}^2$$

For milks poor in fat (skimmed milk) the formula is, in addition: $-0.05 \frac{\text{specific gravity}^2}{\text{solids}} 2.5$.

Detection ot Adulterants.—*Water* is determined by estimating the total solids. Normal milk has, on the average, the following total solid content (from Leffmann after D'Hout):

	Gs. per 100 gs. milk.
Whole milk	14.1
Skimmed milk	9 6
Separator milk	8.0
Cream (22 per cent.)	26.98
Buttermilk	10.0

Starch is detected by acidulating the milk with a drop or two of glacial acetic acid and adding 1 per cent. iodin solution in 80 per cent. ethanol.

[1] If oxidation by means of the acid mixture does not occur promptly, add 2 drops of 30 per cent. H_2O_2 solution and boil again. If still the reaction is not obtained, add 2 more drops of the peroxid.

[2] In each case the last *two figures of specific gravity*, as in Long's coefficient (page 694), are used in place of the whole of the specific gravity figure.

Sodium carbonate (washing soda) is detected by diluting 1 volume of milk with 1 of ethanol 95 per cent. and adding 5 drops of 1 per cent. solution rosolic acid, whereupon a positive test is obtained if a rose color appears. Pure milk gives a brownish-yellow color.

Preservatives.—Formaldehyde may be determined by means of a modified Bial test: (Sumner's method of detection by orcinol). Make a control test: To 2 mgs. formalin solution, diluted with 2 mls. of distilled water, add 10 drops of the following reagent of orcinol. Reagent: Add 6 gs. orcinol to 200 mls. distilled water and add, in addition, 40 drops 10 per cent. ferric chlorid solution. Add to the formalin solution, likewise, 2 mls. concentrated hydrochloric acid. Note the white precipitate. Repeat, using 0.2 mg. of formalin: The precipitation occurs only slowly, but is accelerated by heating. To distinguish acetaldehyde: Acetaldehyde gives similar reactions, but not in dilute solutions. Second, heating the formaldehyde precipitate in its solution for fifteen minutes in the boiling water-bath causes a brownish appearance, which turns to pink when an excess of alkali (NaOH solution) is added. On the contrary, on heating the precipitate of the acetaldehyde solution similarly, there is no brown color and alkali produce a yellow color. A low concentration of formalin gives a greenish fluorescence to the solution, which is not obtained with acetaldehyde.

References: Sumner, J. B., Proc. Soc. Exp. Biol. Med., vol. 20, page 1, 1923. Also: Jour. Amer. Chem. Soc., vol. 45, page 2378, 1923.

Formaldehyde may also be determined, but less satisfactorily, with a bismuth mirror test: Apply Nylander's bismuth method (which does not respond to lactose readily): To 5 mls. of the whole milk add 5 mls. of distilled water and a drop of 10 per cent. NaOH solution. To the diluted solution of milk add 1 ml. of Nylander's solution.[1] Boil gently for about two minutes; a positive test is indicated (but the presence of formaldehyde is not absolutely assured) if the milk becomes dark brown or black, and especially if a mirror of reduced bismuth is found applied to the inside and bottom of the tube after the contents are poured out.

Salicylic acid (and other salicylates): To 25 mls. of milk add Millon's solution in order to precipitate the protids and fats and filter. Add one volume of a mixture of equal parts of ethyl ether and gasolene; shake well, then let the ether-gasolene layer separate. Decant this into a white evaporating dish and evaporate over an electric hot plate or steam bath, *avoiding flames*. To the residue add 1 drop ferric chlorid solution; a violet color is positive.

Boiling of milk is detected by means of the destruction of a peroxidase (page 118) and consequent negative test when peroxidase is sought in a sample of milk: Place 5 mls. of milk in a test-tube and add 1 ml. of a diluted H_2O_2 solution,[2] then 1 ml. of a solution of "Lysol" diluted 1 ml. to 100 mls. with water. A yellow color[3] will appear if the milk is unboiled, but if boiled, a negative reaction.

For use of the Van Slyke CO_2 method see Jour. Biol. Chem., vol. 42, page 41, 1920, article by L. L. Van Slyke (New York Agricultural Station, Geneva, N. Y. Dr. L. L. Van Slyke is father of D. D. Van Slyke).

[1] By means of heat dissolve 2 gs. bismuth subnitrate or subcarbonate, $(Bi(OH)_2NO_3)$; 2 $(BiO_2)CO_3.H_2O)$, 4 gs. Rochelle salt and 100 mls. of 10 per cent. NaOH or KOH solution.

[2] Dilute a commercial 3 per cent. peroxid: 5 mls. of the H_2O_2 diluted with 30 mls. of distilled water.

[3] This test cannot well be done in the yellow light of the ordinary electric illumination; the yellow is never pronounced and is drowned out by the yellow of the light.

TABLE OF MAGNIFICATIONS

Tube length = 160 mms. Projection distance = 250 mms. E. F. = equivalent focus.

Achromatic objectives.		With Huygenian oculars.				
E. F. in millimeters.	Initial magnification.	5×	6.4×	7.5×	10×	12.5×
48	2	10×	13×	15×	20×	25×
32	4	20×	26×	30×	40×	50×
16	10	50×	64×	75×	100×	125×
8	20	100×	130×	150×	200×	260×
4	43	215×	275×	320×	430×	560×
3	57	285×	365×	420×	570×	740×
1.9	95	475×	610×	720×	950×	1260×

TABLES FOR METABOLIC DETERMINATIONS

AVERAGE CALORIES PER HOUR PER SQUARE METER OF BODY SURFACE (DU BOIS)[1]

Age, years.	Males, Calories.	Females, Calories.
14–16	46.0	43.0
16–18	43.0	40.0
18–20	41.0	38.0
20–30	39.5	37.0
30–40	39.5	36.5
40–50	38.5	36.0
50–60	37.5	35.0
60–70	36.5	34.0
70–80	35.5	33.0

DIRECTIONS FOR USE OF FOLLOWING TABLES

Harris-Benedict:[2] The predicted Calories per hour are obtained by adding the Calories corresponding to the weight in kilograms (A) to the Calories corresponding to age and stature (B, C, or D).

Benedict Standards for Girls (E) twelve to eighteen: The predicted Calories are given per kilogram of body weight per hour according to age.

Dreyer:[3] The predicted Calories are based on weight and age (F). Greater accuracy may be obtained by correcting the value for weight according to Dreyer's method. For females, subtract 7 to 10 per cent.

Interpolation: In the use of these condensed tables interpolation is necessary only for critical cases; ordinarily, for clinical work, it is unnecessary.

[1] Page 24.

[2] Benedict, F. G. (page 23). Harris, J. A., biometrist, Carnegie Institution, Cold Springs Harbor, Long Island, N. Y.

[3] Dreyer, G., Professor of Pathology, Oxford University, England.

A: Harris-Benedict Standards Based on Body Weight

Weight, kilograms.	Calories per hour: Males.	Females.	Weight, kilograms.	Calories per hour: Males.	Females.	Weight, kilograms.	Calories per hour: Males.	Females.
10	8.5	....	52	32.6	48.0	94	56.6	64.8
12	9.7	....	54	33.7	48.8	96	57.8	65.6
14	10.8	....	56	34.9	49.6	98	58.9	66.4
16	12.0	....	58	36.0	50.4	100	60.1	67.2
18	13.1	....	60	37.2	51.2	102	61.2	68.0
20	14.3	....	62	38.3	52.0	104	62.4	68.8
22	15.4	....	64	39.5	52.8	106	63.5	69.6
24	16.6	....	66	40.6	53.6	108	64.7	70.4
26	17.7	37.6	68	41.8	54.4	110	65.8	71.2
28	18.8	38.4	70	42.9	55.2	112	67.0	72.0
30	19.9	39.2	72	44.0	56.0	114	68.1	72.8
32	21.1	40.0	74	45.2	56.8	116	69.3	73.6
34	22.2	40.8	76	46.3	57.6	118	70.4	74.4
36	23.4	41.6	78	47.5	58.4	120	71.6	75.2
38	24.5	42.4	80	48.6	59.2	122	72.7	76.0
40	25.7	43.2	82	49.7	60.0	124	73.9	76.8
42	26.8	44.0	84	50.9	60.8	126	75.0	77.6
44	28.0	44.8	86	52.0	61.6	128	76.1	78.4
46	29.1	45.6	88	53.2	62.4	130	77.2	79.2
48	30.3	46.4	90	54.3	63.2			
50	31.4	47.2	92	55.5	64.0			

B: Harris-Benedict Standards Based on Age and Stature—Men

Cms.	Years: 20.	25.	30.	35.	40.	45.	50.	55.	60.	65.	70.
150	25.6	24.2	22.8	21.4	20.0	18.6	17.2	15.8	14.4	13.0	11.6
155	26.6	25.2	23.8	22.4	21.0	19.6	18.2	16.8	15.4	14.0	12.6
160	27.7	26.3	24.9	23.5	22.1	20.7	19.3	17.9	16.5	15.1	13.7
165	28.7	27.3	25.9	24.5	23.1	21.7	20.3	18.9	17.5	16.1	14.1
170	29.8	28.4	27.0	25.6	24.2	22.8	21.4	20.0	18.6	17.2	15.8
175	30.8	29.4	28.0	26.6	25.2	23.8	22.4	21.0	19.6	18.2	16.8
180	31.9	30.4	29.1	27.6	26.2	24.8	23.4	22.0	20.6	19.2	17.8
185	32.9	31.5	30.1	28.7	27.3	25.9	24.5	23.1	21.7	20.3	18.9
190	34.0	32.5	31.2	29.7	28.3	26.9	25.5	24.1	22.7	21.3	19.9
195	35.0	33.6	32.2	30.8	29.4	28.0	26.6	25.2	23.8	22.4	21.0
200	36.1	34.6	33.2	31.8	30.4	29.0	27.6	26.2	24.8	23.4	22.0

C: Harris-Benedict Standards Based on Age and Stature—Women

Cms.	Years: 20.	25.	30.	35.	40.	45.	50.	55.	60.	65.	70.
150	7.7	6.7	5.7	4.7	3.8	2.8	1.8	0.9	0.0	—1.0	—2.0
155	8.1	7.1	6.1	5.1	4.2	3.2	2.2	1.2	0.2	—.7	—1.7
160	8.5	7.5	6.5	5.5	4.5	3.6	2.6	1.6	0.6	—.3	—1.3
165	8.8	7.8	6.9	5.9	4.9	4.0	3.0	2.0	1.0	0.0	—.9
170	9.2	8.2	7.3	6.3	5.3	4.3	3.4	2.4	1.4	0.5	—.5
175	9.6	8.6	7.6	6.7	5.7	4.7	3.7	2.8	1.8	0.8	—.2
180	10.0	9.0	8.0	7.0	6.1	5.1	4.1	3.2	2.2	1.2	0.2
185	10.4	9.4	8.4	7.5	6.5	5.5	4.5	3.5	2.6	1.6	0.6
190	10.8	9.8	8.8	7.8	6.8	5.9	4.9	3.9	3.0	2.0	1.0
195	11.2	10.2	9.2	8.2	7.2	6.2	5.3	4.3	3.3	2.4	1.4
200	11.5	10.5	9.6	8.6	7.6	6.7	5.7	4.7	3.7	2.7	1.0

D: Harris-Benedict Standards Based on Age and Stature—Boys

Cms.	Years: 10.	15.	20.	Cms.	Years: 10.	15.	20.
100	18.0	16.6	15.2	155	29.5	28.1	26.6
105	19.0	17.7	16.3	160	30.5	29.1	27.7
110	20.0	18.7	17.3	165	31.5	30.1	28.7
115	21.0	19.7	18.3	170	32.6	31.2	29.8
120	22.1	20.8	19.4	175	33.6	32.2	30.8
125	23.2	21.8	20.4	180	34.7	33.3	31.9
130	24.2	22.9	21.5	185	35.7	34.3	32.9
135	25.3	23.9	22.5	190	36.8	35.4	34.0
140	26.3	25.0	23.6	195	37.8	36.4	35.0
145	27.4	26.0	24.6	200	38.9	37.4	36.1
150	28.4	27.0	25.6				

E: Benedict Standard for Girls Twelve to Eighteen

Calories per kilogram per hour from age			
Age.	Calories per hour.	Age.	Calories per hour.
12	1.29	15	0.99
12.5	1.24	15.5	0.94
13	1.19	16	0.91
13.5	1.14	16.5	0.91
14	1.09	17	0.91
14.5	1.04	18	0.91

F: Dreyer Standards for Men

Kilos.	Years: 15.	20.	25.	30.	35.	40.	45.	50.	55.	60.	65.	70.	75.	80.
25	45.2	43.5	42.3	41.2	40.4	39.7	39.0	38.5	38.0	37.5	37.1	36.8	36.5	36.2
30	49.5	47.6	46.3	45.2	44.2	43.5	42.8	42.2	41.7	41.2	40.7	40.3	39.8	39.6
35	53.5	51.5	50.0	48.8	47.8	46.9	46.2	45.6	45.0	44.5	44.0	43.6	43.2	42.8
40	57.1	55.0	53.5	52.1	51.1	50.2	49.4	48.8	48.1	47.5	47.0	46.6	46.2	45.8
45	60.1	58.4	56.7	55.3	54.2	53.3	52.4	51.7	51.1	50.5	50.0	49.5	49.0	48.6
50	64.9	61.6	59.7	58.4	57.1	56.1	55.3	54.5	53.8	53.2	52.6	52.1	51.6	51.2
55	66.7	64.6	62.7	61.2	59.9	58.9	58.0	57.2	56.4	55.8	55.2	54.6	54.1	53.7
60	70.1	67.5	65.5	63.9	62.6	61.5	60.6	59.7	58.9	58.3	57.7	57.0	56.6	56.1
65	72.5	70.2	68.1	66.5	65.1	64.0	63.0	62.2	61.4	60.6	60.0	59.5	58.9	58.3
70	75.6	72.9	70.7	69.0	67.7	66.4	65.4	64.5	63.7	63.0	62.2	61.6	61.1	60.5
75	78.3	75.4	73.3	71.4	70.0	68.7	67.7	66.8	65.9	65.2	64.5	63.8	63.3	62.7
80	80.9	77.9	75.6	73.8	72.3	70.0	69.9	68.9	68.0	67.2	66.5	65.9	65.3	64.7
85	83.4	80.3	78.0	76.1	74.5	73.2	72.1	71.0	70.2	69.3	68.6	67.9	67.3	66.7
90	85.7	82.6	80.2	78.1	76.7	75.3	74.1	73.1	72.2	71.3	70.6	69.9	69.3	68.7
95	88.1	84.4	82.3	80.4	78.7	77.4	76.2	75.1	74.2	73.3	72.4	71.8	71.2	70.5
100	90.4	87.1	84.4	82.5	80.8	79.4	78.2	77.0	76.1	75.2	74.4	73.7	73.0	72.4
105	92.5	89.2	86.6	84.5	82.8	81.3	80.0	79.0	77.5	77.0	76.3	75.5	74.8	74.2
110	94.8	91.4	88.6	86.4	84.8	83.3	82.0	80.9	79.8	78.9	78.1	77.3	76.6	75.9
115	97.0	93.4	90.7	88.6	86.7	85.2	83.9	82.6	81.6	80.7	79.8	79.0	78.2	77.6
120	99.1	95.4	92.7	90.3	88.6	87.0	85.7	84.4	83.3	82.4	81.5	80.7	79.9	79.3

BENEDICT-TALBOT[1] STANDARDS FOR BOYS AND GIRLS

Calories per hour from body weight					
Weight, kilograms.	Boys, Calories.	Girls, Calories.	Weight, kilograms.	Boys, Calories.	Girls, Calories.
3	6.3	6.3	31	47.5	44.6
4	8.8	9.2	32	48.3	45.4
5	11.3	11.9	33	49.2	46.4
6	13.8	14.6	34	50.0	47.3
7	16.3	16.9	35	50.8	48.3
8	18.5	19.2	36	51.7	49.2
9	20.6	20.8	37	52.3	50.1
10	22.7	22.5	38	53.1[2]	50.7
11	24.6	24.2	39		50.8
12	26.0	25.4	40		51.0
13	27.5	26.7	41		51.2
14	29.0	27.7	42		51.3
15	30.2	28.8	43		51.4
16	31.5	29.6	44		51.6
17	32.5	30.6	45		51.7
18	33.5	31.7	46		51.8
19	34.6	32.5	47		52.0
20	35.8	33.5	48		52.1
21	36.9	34.6	49		52.3
22	37.9	35.6	50		52.4
23	39.2	36.7	51		52.5
24	40.2	37.5	52		52.7
25	41.3	38.8	53		52.8
26	42.5	39.6	54		52.9
27	43.5	40.6	55		53.1
28	44.6	41.7	56		53.2
29	45.4	42.5	57		53.3
30	46.5	43.5	58		53.5

[1] Talbot, F. B., Harvard Med. School, Boston, Mass.
[2] For boys apply Harris-Benedict Standards: A + D.

VITAMINS

COWGILL'S[1] EXPRESSION FOR VITAMIN B REQUIREMENT

$$K_{\text{vitamin}} = \frac{\text{Vitamin B per twenty-four hours}}{\text{Cals. per twenty-four hours} \times (\text{body weight})^{\frac{2}{3}}}$$

[1] Cowgill, G. R., Smith, A. H., and Beard, H. H. (Yale University), Jour. Biol. Chem., vol. 63, p. xxiii, 1925.

VITAMINS

(Distribution Among Common Foods)

	Vitamin A.	Vitamin B.	Vitamin C.	Vitamin D.	Vitamin E.
Apple	P	PP	PP		
Banana	?	P	P		
Barley (unsprouted)	P	PP	...	P[1]	(sprouted)
Beans, kidney	...	PPP	...	...	(sprouted)
Soy	P	PPP	a	...	(sprouted)
Beet	a	P	P	P[1]	
Bile (beef)	...	...	...	P[1]	
Brain substance	P	PP	?	P	P
Bread, highly milled flour, water	a	P	a	a	a
Whole wheat	P	PP	a	a	a
Milk	P	P	?	P	
Butter	PPP	a	a	(fresh)	P
Cabbage (raw)	PP	PPP	PPP	...	?
Carrots	PP	P	P		
Cauliflower	P	PP	?		
Celery	?	PP	?		
Chard	P	P	?		
Cheese	PP	PP	a	P[1]	slight
Cocoanut oil (butter substitute)	a	a	a	P	a
Cod-liver oil (undecomposed)	PPP	a	a	PP	P
Corn oil (yellow corn)	?	a	a	P	PP
Cottonseed oil	?	a	a	(when irradiated)	P[2]
Cream	PPP	P	?	(when irradiated)	P[3]
Cucumber	...	P			
Greens (dandelion)	PP	PP	P	P	P
Egg (whole)	PPP	P	a	P	P
Fat (suet)	P	?	?	?	?
Fish (lean fat)	a	P			
(fat, like mackerel)	P	P	...	a	
Germinated beans, etc.	P	PP	PPP	P	PP
Grains	P	a	a	P[1]	
Grapefruit	?	PP	PP	a	a
Grapejuice (trade brands)	?	P	P	a	a
Grapes, fresh	?	P	P	a	
Heart substance	P	P	P	P[3]	P
Hemp seed	P	...	...	...	PP
Honey	a	P	a	a	
Kidney	PP	PP	P	P	P
Lanolin	...	...	...	P[1]	
Lard	a	a	a		
Lemon juice	a	PP	PPP		

[1] Irradiated.

[2] Fertility good, but lactation indifferent.

[3] From milk of cows on fresh pasturage.

VITAMINS (continued)

(Distribution Among Common Foods)

	Vitamin A.	Vitamin B.	Vitamin C.	Vitamin D.	Vitamin E.
Lime (whole)	a	?	PP		
Linseed oil	P	...	...	P[1]	a
Lettuce (grown in open air)	PP	PP	PPP	P	P
Liver tissue	PP	PP	?	P	P
Meat (steak, chops)	a	slight	P[2]	a	P[3]
Milk, fresh, unpasteurized	PPP	PP	PP	PP	PP
Condensed	PP	P	P[6]	P[2]	
Dried, whole milk	PPP	PP	P[7]		
Skimmed	P	P	P[2]		
Nut oils	...	...	a	P[1]	P
Oatmeal, from cracked oat	P	PP	a	a	
Oleomargarin, animal and vegetable fats	P	a	a	P[1]	P[3]
Olive oil	a	a	a	P[1]	P[3]
Onion	...	PP	PP		
Orange juice	P	PP	PPP		
Pancreas juice	a	PPP	?	P	P
Parsnips	a	PP			
Peach kernel oil	P	...	...	...	P[2]
Peanut oil	P	PP	...	...	P
Peas, fresh	P	PP	PPP	P	
Potato, sweet	PP	P			
White, boiled	P	PP	PP		
Prunes	a	P	a		
Rice, polished (also "red" or "brown")	a	a	a	a	a
Roe (fish-eggs)	P	PP	?	P	P
Rye, cracked	P	PP			
Sesame oil	P	...	...	..,	a
Shark and burbot oils	PP	a	a	P[3]	P
Soy-bean oil	P	...	...	...	P[2]
Spinach[8]	PPP	PPP	PPP	P[1]	
Squash	PP	...	...	...	P[4]
Sweetbread thymus	a	a	a	...	P?
Tea, etc.	a	a	a	a	a
Tomato	PP	PPP	PPP		
Wheat embryo	PP	PPP	a	slight	PP (*Most potent*)
Whey (from milk)	?	PP	?	...	PP[5]
Yeast	a	PPP	a	P[1]	

[1] Irradiated. [2] From milk of cows on fresh pasture.

[3] Fertility good, but lactation indifferent. [4] Seeds.

[5] Ferris citrate added.

[6] Vitamin preserved by conserving action of sugar.

[7] Human milk is now dried for infant feeding; about one fourth of vitamin C content is lost.

[8] Only that grown in the open in summer, that grown during spring, autumn or winter is without vitamin A value.

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